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The Department of Research Medicine of the
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VOLUME VII

1922–1923

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Reprinted from The American Journal of Physiology
Vol. 58, No. 3, January, 1922

BLOOD FIBRIN STUDIES

I. An Accurate Method for the Quantitative Analysis of Blood Fibrin in Small Amounts of Blood

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From the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco

Received for publication July 19, 1921

Our present methods for the quantitative estimation of fibrin or fibrinogen are not satisfactory. The amount of blood required for single determination is large, so much so that determinations repeated at short intervals will at once introduce the factor of anemia. Many methods are relatively inaccurate and time consuming. Meek gives an excellent review of the development of these various methods (1).

The proposed method is quite satisfactory in our hands and enables a laboratory worker to estimate the fibrin content of the blood plasma accurately and with little expenditure of time. The amount of whole blood required is 8 to 10 cc. We estimate the *fibrin in whole blood* by determining the red cell hematocrit. Both values are given in all our experiments—the fibrin per 100 cc. plasma and per 100 cc. whole blood.

Previous workers have isolated fibrin or fibrinogen from the other blood elements in the following ways:

1. The blood sample was allowed to clot spontaneously. The fibrin was then freed from the other blood elements by grinding the clot and washing with water, alcohol and ether. The residue of fibrin was subsequently dried and weighed. The experimental error of this method is very large. It is practically impossible to wash the fibrin free from the other blood elements and in grinding and washing some of the fibrin may be lost. The amount of blood necessary for a relatively accurate analysis is large (50 to 100 cc.).

2. Spontaneous coagulation was inhibited by mixing the blood sample with a 3 per cent sodium fluoride solution. The formed elements were then allowed to settle, or were thrown down by the centrifuge. The fibrinogen was then precipitated from the plasma by the proper concentration of ammonium sulfate. This precipitate was washed thoroughly with water, alcohol and ether, then dried and weighed(2). This method is an improvement over the first in that the formed elements of the blood no longer complicate the analysis. The salting out process is not satisfactory, as it is well known that the various plasma proteins do not precipitate uniformly at a given salt concentration. Filtration of serum is a slow process and almost always involves a loss of material. Again, at least 50 cc. of blood must be used for a single analysis.

3. Spontaneous coagulation was inhibited by mixing the blood sample with sodium oxalate solution. The plasma obtained by centrifugalization was made faintly acid with 5 per cent acetic acid and heated for 10 to 15 minutes at 58° to 60°C. to precipitate the fibrinogen. The precipitate was separated by the centrifuge, washed, dried and weighed (3). The heat precipitation method of Whipple and Hurwitz (3) is also open to criticism. The heating may coagulate other of the blood proteins than fibrinogen, particularly if the acidity is not properly adjusted. It is known that a plasma very low in fibrinogen content may give little if any precipitate by this heat method—in other words, there is a serious error as we approach low values of blood fibrinogen. It seems evident from some experiments tabulated below, that this heat method is quite accurate when large quantities of plasma are used (25 cc.) and when the fibrinogen content of the blood is within normal limits.

4. Spontaneous coagulation was inhibited by mixing the blood sample with sodium oxalate solution. The plasma obtained by centrifugalization was diluted with 0.8 per cent sodium chloride solution and the fibrin coagulated by the addition of calcium chloride solution. A Kjeldahl determination on the washed fibrin gives the weight of fibrin in the sample of plasma used (4). This method developed by Cullen and Van Slyke (4) is a step toward greater accuracy. The dilution of the plasma permits of easy isolation of the fibrin. Ten cubic centimeters of plasma are sufficient for an analysis if the fibrin content of the blood is normal or above normal. If the fibrin content is below normal a larger amount should be used.

DEVELOPMENT OF THE METHOD. The blood was prevented from clotting by mixing with sodium oxalate solution. The centrifugalization was done in a graduated 15 cc. hematocrit tube in order that the per cent of formed and fluid elements might be accurately determined. The analysis was made from a sample of plasma diluted with salt solution to facilitate the separation of the fibrin from the other blood proteins. The fibrin was coagulated by recalcification of the dilute mixture. The presence of the calcium oxalate formed by the addition of calcium chloride solution to the oxalated plasma made impossible the direct weighing of the dried fibrin thus obtained. The sample of fibrin was dried to a constant weight. The fibrin was then ignited in a crucible which was then reweighed to determine the amount burned off. The residue of calcium oxide was thus corrected for.

TABLE 1

Variation in amount of oxalate solution in this method; dog 17–160; condition, normal

SODIUM OXALATE 1 PER CENT SOLUTION	PLASMA	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN		FIBRIN	
				Net	Average	100 cc. Plasma	100 cc Blood
cc.	*cc.*	*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1.0	2 2	9.1	54	6.6 7.0	6.8	420	195
1.5	2 2	9.0	56	7.0 7.0	7.0	433	189
2.0	2 2	9.0	56	6.8 7.1	6.95	425	186
2.5	2 2	9.4	59	7.5 7.7	7.6	460	187
3.0	2 2	9.0	63	7.5 7.5	7.5	456	170

It seemed desirable to control a number of factors in this method.

1. We intended to use a minimum amount of *sodium oxalate*. The solution was usually 1.0 per cent, although it is recognized that this is slightly hypotonic. The use of 1 cc. of a 1.0 per cent solution with 9 cc. blood is close to the limit of safety. The addition of a little more blood to this mixture will often start blood coagulation. Table 1 shows the reaction due to increasing amounts of sodium oxalate solution (1.0 per cent or hypotonic.) The red cell hematocrit shows a progressive increase with increasing amounts of sodium oxalate solution per unit amounts of blood. The figures for fibrin per 100 cc. whole blood are

relatively constant up to the last dilution where the great excess of oxalate may have modified the blood coagulation.

2. It has been shown in this laboratory (6) that *centrifugalization* of oxalated dog's blood for 30 minutes at a speed of three thousand revolutions per minute will completely express all plasma from between the packed cells. This procedure will give uniform results and an accurate measure of the ratio of blood cells and plasma. From such observations it is easy to compute the relative amounts of red cells or plasma in per cent and the contained fibrin in plasma or whole blood. The separation is effected in calibrated 15 cc. centrifuge tubes graduated to $\frac{1}{10}$ of 1 cc.

TABLE 2

Dilution of clot by salt solution; dog 19–76; condition, normal

SODIUM CHLORIDE 0.8 PER CENT SOLUTION	PLASMA	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN		FIBRIN	
				Net	Average	100 cc. Plasma	100 cc. Blood
cc.	*cc.*	*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
40 40	2 2	9.2	59	8.2 8.1	8.15	514	213
30 30	2 2	9.2	59	8.0 8.4	8.2	517	214
20 20	2 2	9.2	59	8.4 8.2	8.3	524	217

3. *Dilution of plasma to facilitate handling of clot.* Cullen and Van Slyke used an 0.8 per cent sodium chloride solution to dilute the plasma. This is a slightly hypotonic solution but when mixed with a small amount of 2.5 per cent calcium chloride solution the resulting mixture closely approaches blood isotonicity. We attempted a few plasma coagulations in distilled water plus calcium chloride solution and the result was a poorly formed amorphous mass. The 0.8 per cent solution plus the 2.5 per cent calcium chloride gave a uniformly good result and was used in all routine experiments.

Table 2 gives the results obtained with the same sample of blood using varying amounts of 0.8 per cent sodium chloride solution plus 2 cc. of 2.5 per cent calcium chloride solution. The smallest amount of salt solution which permits easy separation of fibrin from the other blood proteins is the optimum amount because it permits easy handling of the sample. Too great dilution weakens the clot to such a degree that bits of fibrin are easily broken off and lost, while too scant a dilution does not make the texture of the clot coarse enough to permit the other blood proteins to be expressed easily and completely.

If 40 cc. of salt solution are used the clot formed from 2 cc. of plasma can be easily and safely manipulated with a glass rod and freed by gentle pressure from the enclosed fluids. Absolutely no part of the fibrin need be lost during this process and the resulting mass of fibrin can then be washed easily with water or salt solution. This simple procedure eliminated filtration which is tedious and often introduces inaccuracies.

4. Some experiments were performed to ascertain the extent of possible dilution. It was suspected that extreme dilution might inhibit the formation of fibrin in vitro under these conditions. We have no evidence that extreme dilution inhibits clot forming. Some experiments carried this dilution beyond ten times the customary dilution but the clot formed as usual. It was difficult to handle these very tenuous clots and errors from loss of material are to be expected under such condition.

Table 3 gives further control of the accuracy of this method and shows that considerable variation in the amount of the calcium chloride solution will not introduce an error. Excessive amounts of this salt may modify the coagulation. We use 2 cc. of a 2.5 per cent solution as a routine.

5. Coagulation does not take place at once on setting up a plasma mixture. Some experiments were done to determine the length of time necessary for complete coagulation, also the length of time the mixture may stand after coagulation without autolysis of the fibrin. It was found that the clotting time varied somewhat when the blood was abnormal. In some peptone injection experiments the blood clotted only after normal serum had been added to the mixture. Other experimental conditions to be reported in a later paper caused the same inhibition of coagulation.

Table 4 gives the results obtained by allowing samples of the same plasma to coagulate and stand at room temperature for different lengths of time. Before the half-hour period the clot was not sufficiently formed to permit manipulation. Uniform analyses were obtained from samples allowed to clot from one to twenty-four hours. After 48 hours the clot was generally autolysed and fragile. Occasionally samples have given normal readings after 48 hours. A clot should not stand more than 24 hours and the best results are obtained by expressing the fluid elements from the clot after 2 or 3 hours. Cullen and Van Slyke recommend 10 to 15 minutes for the clotting of the recalcified plasma. In our experience this time is too short and may lead to error, at least in plasmata obtained from animals and abnormal human beings.

TABLE 3

Variations in the amounts of calcium chloride used; dog 19–45; condition, normal

CALCIUM CHLORIDE 2.5 PER CENT SOLUTION	PLASMA	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN		FIBRIN	
				Net	Average	100 cc. Plasma	100 cc. Blood
cc.	*cc.*	*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
2 2	2 2	9.1	44	14.3 14.3	14.3	855	480
3 3	2 2	9.1	44	14.6 14.6	14.6	873	489
4 4	2 2	9.1	44	14.4 14.2	14.3	855	480
5 5	2 2	9.1	44	13.8 14.4	14.1	843	473
6 6	2 2	9.1	44	14.1 14.2	14.15	846	475
7 7	2 2	9.1	44	14.1 14.2	14.15	846	475
8 8	2 2	9.1	44	13.9 13.6	13.75	822	461
9 9	2 2	9.1	44	13.0 13.6	13.3	795	446
10 10	2 2	9.1	44	13.8 14.0	13.9	831	446

In abnormal blood when clotting does not take place after recalcification coagulation will occur if 5 to 10 cc. of fresh serum are added. The usual method may be followed after that and good results obtained. In such samples a longer time for clotting must be allowed.

6. May oxalated blood stand for any length of time, and still be suitable for analysis of fibrin content? Table 5 shows the results of

experiments on the same sample of blood preserved 24 hours at room temperature and in the ice-box (3 to 7°C.). It is obvious that normal blood may stand either in the ice-box or at room temperature for 24 hours and still be suitable for a fibrin analysis. Bloods taken from cases of suspected liver injury should be analyzed at once as the tendency for autolysis in these cases is markedly increased. Our observations confirm those of Goodpasture (5) in reference to such cases.

TABLE 4

Optimum time for coagulation; dog 19–76; condition, normal

COAGULATION TIME	PLASMA	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN		FIBRIN	
				Net	Average	100 cc. Plasma	100 cc. Blood
hours	*cc.*	*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
½*							
1	2 2	9.2	59	8.2 7.8	8.0	505	209
1½	2 2	9.2	59	7.9 7.8	7.85	495	205
2	2 2	9.2	59	7.9 8.0	7.95	502	208
2½	2 2	9.2	59	8.0 8.4	8.2	517	214
24	2 2	9.2	59	8.0 8.0	8.0	505	209
48	2 2	9.2	59	†			

* Before 30 minutes clot too soft to handle.

† Clot autolysed.

Room temperature about 20°C.

7. As has been stated, the main objection to the existing methods for fibrin analysis was the fact that large quantities of blood were necessary for an accurate analysis. We wished to find the smallest amount of plasma that could be used and still give an accurate reading Cullen and Van Slyke (4) obtained uniform results with 10 cc. samples. We were interested to know whether smaller amounts could be used with accurate results. Accordingly, a number of analyses was made on the

same sample of blood using different amounts of plasma. Table 6 is a sample of a very large number of determinations made to establish this point. A 2 cc. sample of plasma is sufficient to give a reliable analysis by this method. Some experiments were carried out with a one cubic centimeter sample of plasma, but the results obtained were somewhat irregular.

8. The time necessary for complete dehydration of the clot in an oven at 110°C. varied with the quantity of fibrin. For the amount usually obtained from a 2 cc. sample of plasma, 3 hours were found to be sufficient. After this period constant weights were obtained for a given specimen. When larger amounts of plasma were used, a longer time was necessary. We encountered no specimens that were not thoroughly dry after 12 hours at 110°C. After weighing the crucible and fibrin contained therein the fibrin was ignited over a free flame. Samples were ignited and weighed at intervals from the time all the carbon had

TABLE 5

Comparison of fresh blood with blood preserved 24 hours; normal dogs

TIME	PLASMA	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN		FIBRIN		Remarks
				Net	Average	100 cc. Plasma	100 cc. Blood	
hours	*cc.*	*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	
Fresh	2 2	10.0	50*	6.4 6.4	6.4	384	192	Dog 19–21 Dec. 30, 1919
24	2			6.5	6.5	390	195	Room temperature
Fresh	2 2	10.0	50*	5.0 5.4	5.2	312	156	Dog 18–60 June 24, 1918
24	2			5.2	5.2	312	156	Room temperature
Fresh	2 2	10.0	50*	6.4 6.6	6.5	390	195	Dog 17–28
24	2			6.5	6.5	390	195	Room temperature
24	2 2			6.5 6.9	6.7	402	201	Ice box 3°–7°C.

* Assumed value for hematocrit to give simple relative values for control and 24 hour specimens.

disappeared up to one hour thereafter. A safe limit to place as the burning time is 15 minutes.

9. One essential control of the method was the serum coagulation in place of the routine coagulation by means of calcium chloride. The use of fresh serum obviates the formation of the calcium oxalate precipitate which is present in the usual reaction and insures a normal clot formation modified only by the presence of the diluent (0.8 sodium chloride) to insure a clot of loose texture. We may say that the results are identical whether the diluted plasma is coagulated by fresh serum or a calcium chloride solution (table 7).

TABLE 6

Comparative readings using varying amounts of plasma; dog 18-124; condition, normal

PLASMA	R. B. C. HEMATOCRIT	FIBRIN		FIBRIN	
		Net	Average	100 cc. Plasma	100 cc. Blood
cc.	*per cent*	*mgm.*	*mgm.*	*mgm.*	*mgm*
10	43	54.2	54.0	638	362
10		53.8			
5		27.8	27.72	655	371
5		27.5			
5		27.9			
5		27.7			
2		11.1	11.1	656	372
2		11.1			

Dried serum was found to be unsuitable as a coagulant. At times, when dealing with abnormal blood, it is necessary to add serum to cause coagulation. If serum could be dried and kept on hand for this purpose, it would obviously be a convenience. Such serum does not act satisfactorily, however, and either fails to cause coagulation, or gives a muddy looking and fragile clot not suitable for analysis. Tissue extracts, which are sometimes used to furnish thromboplastin and promote coagulation, act much like dried serum and do not form a coagulum satisfactory for analysis. A fairly large amount of serum is necessary to cause complete coagulation of a sample of plasma. We found it necessary to add at least twice as much fresh serum as plasma to insure complete coagulations.

Control analyses were made on the same blood samples by the Kjeldahl method of Cullen and Van Slyke (4), the heat coagulation method of Whipple and Hurwitz (3) and the method here described to test the accuracy of results obtained by these methods and to find the limitations of each method. For our own satisfaction a great many analyses were made, but we will present here only three tables. It will be noted that the tables 8, 9 and 10 present blood samples of high fibrinogen content, normal and low fibrinogen content.

TABLE 7

Coagulation value of serum and calcium chloride compared; normal and abnormal dogs

CALCIUM CHLORIDE 1 PER CENT SOLUTION	FRESH SERUM	PLASMA	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN		FIBRIN		REMARKS
					Net	Average	100 cc. Plasma	100 cc. Blood	
cc.	*cc.*	*cc.*	*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	
5		5	9.2	37	46.8	47.0	1100	693	Dog 18–8
5		5			47.2				High fibrin
	15	5			46.7	46.45	1089	686	Peritonitis
	15	5			46.2				
2		2	9.2	37	17.9	18.35	1076	678	
2		2			18.8				
	5	2			18.3	18.35	1076	678	
	5	2			18.4				
2		2	8.85	49	6.5	6.45	395	201	Pup 3 A
2		2			6.4				
	5	2			6.8	7.1	435	222	
	5	2			7.4				
5		5	9.1	61	31.8	31.6	807	315	Dog 19–21
5		5			31.4				
	10	5			31.5	31.1	794	310	Fibrin above normal
	10	5			30.8				

With normal blood (table 8) all of the estimations, except the 2 cc. samples by the Kjeldahl method, gave corresponding readings. The two cubic centimeter samples with the Kjeldahl give a reading that was high. It will be noted that this was true for all these experiments.

It will also be noted in table 9 (high fibrin content) that the result by the Kjeldahl and the dry weight methods checked within reasonable limits. The heat coagulation method gave a result that was too high. This was probably due to the difficulty of washing such a large bulk of precipitate free from the other blood proteins. The Kjeldahl estimations on the 5 and 10 cc. samples were within the expected limits of accuracy but the estimation with 2 cc. of plasma gave a high reading. Both the 5 cc. and the 2 cc. samples by the dried weight method gave accurate readings.

TABLE 8

Comparative test of methods; normal dog; normal fibrin content

PLASMA	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN		FIBRIN		REMARKS
			Net	Average	100 cc. Plasma	100 cc. Blood	
cc.	*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	
5 5	9.1	58	17.7 17.9	17.8	449	188	Dry weight method
2 2	9.1	58	7.3 7.2	7.25	457	191	
10 10	9.1	58		32.6	411	171.8	Kjeldahl method
5 5	9.1	58		15.4	388	162	
2 2	9.1	58		7.9	498	208.2	
25 25	9.1	58	85.5 86.5	86.0	434	181.4	Heat coagulation method

The greatest discrepancies between the three methods arose when a blood with a *low fibrin content* was analyzed (table 10). By the heat coagulation method not enough fibrinogen was precipitated to permit of analysis. The Kjeldahl method gave accurate results with 10 and 5 cc. amounts, but again gave a high reading with a 2 cc. amount of plasma. The dry weight method again gave uniform results with all amounts. From these experiments we were led to conclude that the dry weight method is more accurrate for small amounts of fibrin than the other methods described.

Technique of the method. Approximately 9 cc. of blood are collected from the vein by needle into a vaselined syringe and delivered immediately into a 15 cc. hematocrit tube which contains 1 cc. of a 1 per cent sodium oxalate solution. The tube with its contents is immediately inverted twice to insure thorough mixing. The mixture is centrifugalized at high speed (3000 revolutions per minute) for 30 minutes. The amount of cellular elements and plasma are read to tenths of a cubic centimeter. Exactly 2 cc. of the plasma are delivered by a calibrated pipette into a tumbler containing 40 cc. of an 0.8 per cent solution of sodium chloride, and 2 cc. of a 2.5 per cent solution of calcium chloride. A similar duplicate sample is set up. The preparations are thoroughly mixed and allowed to stand at room temperature for 2 hours. To some abnormal blood plasmas it is necessary to add normal serum and a longer period may be required to insure complete coagulation.

TABLE 9

Comparative test of methods; dog; distemper and pneumonia; high fibrin content

PLASMA	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN		FIBRIN		REMARKS
			Net	Average	100 cc. Plasma	100 cc Blood	
cc.	*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	
5 5	10.0	33	54.2 54.3	54.25	1246	835	Dry weight method
2 2	10.0	33	21.4 22.0	21.7	1246	835	
10 10	10.0	33		112	1287	858.3	Kjeldahl method
5 5	10.0	33		55	1231	821	
2 2	10.0	33		23.9	1373	919.9	
25 25	10.0	33	304.6 306.6	305.9	1406	942	Heat coagulation method

The fibrin is freed from the fluid elements by gentle manipulation and pressure with a glass rod. The fibrin mass thus obtained is then washed in distilled water. The clear free fibrin is then placed in a small porcelain crucible and dried in an oven at 110°C. to a constant weight. This requires from 3 to 10 hours, depending on the amount of fibrin. After drying, the crucible is placed in a desiccator and allowed to cool. The weight of the crucible is recorded to tenths of a milligram. The fibrin is then ignited in the crucible over a Bunsen flame (15 minutes combustion time). While still warm the crucible is placed in a desiccator. When cool it is reweighed. The difference in the weights before and after burning is the weight of fibrin for the sample analyzed. Knowing the weight of fibrin per cubic centimeter of plasma and the ratio of red blood cells to plasma, we may calculate the amount of fibrin in 100 cc. of whole blood or blood plasma.

TABLE 10

Comparative test of methods; dog 18-87; chloroform poisoning; low fibrin content

PLASMA	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN		FIBRIN		REMARKS
			Net	Average	100 cc. Plasma	100 cc. Blood	
cc.	*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	
5 5	9.2	47	4.0 4.0	4.0	92	49.0	Dry weight method
2 2	9.2	47	1.8 1.5	1.65	99	53	
10 10	9.2	47		7.28	87	46.4	Kjeldahl method
5 5	9.2	47		3.73	89	47.4	
2 2	9.2	47		2.42	145	77.3	
25 25	9.2	47			Mere trace		Heat coagulation method

SUMMARY

A simple and accurate method for the quantitative analysis of blood fibrin is outlined. Blood plasma obtained from oxalated blood is coagulated slowly in a dilute solution and the delicate thin-meshed clot carefully washed, dried and weighed. Combustion and re-weighing of

residue gives the correct figures for the dry fibrin from which can be computed the plasma fibrin content. All determinations are made in duplicate. With accurate hematocrit readings the fibrin content of whole blood may then be estimated.

Various steps in this method are carefully controlled in the tabulated experiments and the optimum conditions are established.

Comparative determinations with other methods show interesting figures. The normal blood plasma may show practically identical figures in fibrin or fibrinogen analyses with all the common methods in use. This method is more accurate than the older methods when small amounts of plasma are used.

Abnormal plasma specimens may show greater differences in fibrin values and this method appears to be more accurate and dependable.

By this method it is possible to analyze blood samples at short intervals of time in a single experiment without introducing the factor of anemia (hemorrhage). The level of blood fibrin may thereby be established and plotted as a curve based on a large number of daily or even hourly observations. That this blood fibrin level may be influenced by a great number of factors will be shown in subsequent communications.

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Reprinted from The American Journal of Physiology
Vol. 58, No. 3, January, 1922

BLOOD FIBRIN STUDIES

II. Normal Fibrin Values and the Influence of Diet

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Received for publication July 19, 1921

Having available an accurate method for fibrin analysis using small amounts of blood we determined to study the normal fluctuations in blood fibrin which have been observed by other workers. By this method it is possible to make accurate fibrin determinations in duplicate using but 8 to 10 cc. of whole blood. It is therefore possible to make daily or even more frequent fibrin determinations in dogs without any complications due to anemia. As one studies the tables given below it is noted that the individual fluctuations in fibrin values are not as great as have been reported by other workers (1). However, under the most rigidly controlled conditions normal dogs show considerable fluctuation in fibrin values and we must look upon the fibrin of the blood plasma as a most labile substance. Compared with the other plasma proteins (2) which are so stable in total concentration the fibrin is truly mercurial in its changes. This may indicate a totally different source of production in the body as well as the greatest difference in general utility. The body under physiological conditions will not tolerate a low blood plasma fibrinogen content and promptly (a few hours) forms sufficient material to return the fibrinogen level to normal or above normal.

Experimental observations. Dogs were used in all these experiments and unless otherwise stated were in excellent physical condition. The daily samples were taken before the food or diet mixture was given. Dogs were fed once daily unless note is made to the contrary. The fibrin method has been fully described in the first paper of this series. All determinations were done in duplicate. An important control experiment is found in table 14, which shows the minimal action which follows daily bleedings. This large dog was bled 40 cc.

every day over a long period with little disturbance of the fibrin level. Smaller bleedings have even less influence on the curve of fibrin values. We feel confident therefore that our method of removing small samples of blood from normal dogs does not in itself modify the curve of fibrin values as determined by subsequent analyses.

The blood fibrin values for normal dogs are given in tables 11, 12 and 13. The average figures for mixed diet are close to 400 mgm. fibrin per 100 cc. plasma. The red cell hematocrit values for normal dogs are slightly less than half the plasma figures.

TABLE 11

Blood fibrin of normal dogs; liberal mixed diet

DATE	DOG		WEIGHT	FIBRIN	
	Number	Type		100 cc. Plasma	100 cc. Blood
			pounds	*mgm.*	*mgm.*
5/16/19	19–104	Pup	17	506	248
12/21/18	15–56	Bull	47	310	140
9/11/18	19–2	Bull	26	351	161
9/11/18	19–21	Pointer	40	306	138
9/11/18	19–1	Bull	40	372	192
12/ 9/18	19–66	Terrier	22	344	150
6/24/19	19–68	Spitz	15	453	226
6/27/19	19–93	Bull	25	462	271
1/22/19	18–30	Bull	35	314	140
12/27/19	16–52	Spitz	20	403	201
6/13/18	18–103	Bull	35	430	159
9/ 5/18	19–124	Mongrel	40	457	228
	18–38	Coach	30	364	182
Maximum				506	248
Minimum				306	140
Variation				200	108
Average				390	187

Fasting conditions give more stability to the blood fibrin values and the physiological fluctuations are less. The average fibrin values are less in fasting than during periods of mixed diet. "Mixed diet" in these papers means a liberal diet of table scraps obtained from the University Hospital, consisting of cooked meat, bones, bread, potatoes, rice, macaroni and vegetables. In certain cases there may be a considerable excess of food given which permits individual selection of food. One dog may prefer meat, or bones or potatoes, etc. The importance of this factor will come out in some later experiments.

It has been stated elsewhere (1) that individual dogs showed less variations in their fibrin values but between different normal dogs the difference in fibrin values might be very considerable. Under uniform conditions we feel that a normal adult dog will not show great fluctuations in blood fibrin values (table 13). It may be safe to say that the physiological variations in the curve of blood fibrin under uniform conditions should not exceed 20 to 30 per cent. Eventually we may hope to understand the factors which cause even these small fluctuations in the blood fibrin values.

TABLE 12

Blood fibrin of normal fasting dogs; food withheld as indicated—water in cage

DATE	DOG		WEIGHT	FIBRIN		REMARKS
	Number	Type		100 cc. Plasma	100 cc. Blood	
			pounds	*mgm.*	*mgm.*	
5/19/19	19–104	Bull	17	376	181	After 3 day fast
5/19/19	17–157	Coach	21	401	219	After 3 day fast
9/23/18	19–2	Bull	26	303	145	After 12 day fast
9/23/18	19–21	Pointer	40	334	164	After 3 day fast
9/23/18	19–1	Bull	40	412	218	After 3 day fast
1/29/19	19–78	Airedale	26	344	130	After 2 day fast
2/ 4/19	19–85	Mongrel	24	398	121	After 2 day fast
2/11/19	19–50	Mongrel		349	184	After 2 day fast
2/25/19	19–6	Mongrel	30	388	152	After 3 day fast
6/30/19	19–124	Mongrel	40	354	157	After 3 day fast
Maximum				412	219	
Minimum				282	121	
Variation				130	98	
Average				358	167	

An important factor is controlled in table 14. Daily bleedings of 40 cc. whole blood removed by hypodermic needle from the jugular vein does not materially disturb the fibrin level in this dog. Presumably as small a dog as ten pounds should tolerate a daily bleeding of 10 cc. without showing serious disturbance of the fibrin or hematocrit values. This dog (table 14) does show a loss in red cell hematocrit per cent but it may be recalled that this rice diet is not favorable for hemoglobin regeneration (3). The general condition remained excellent throughout the long experiment. There is a slight tendency for the fibrin values to rise during the week following the initiation of the daily bleedings. With considerable hemorrhage, this tendency may become quite marked and may carry the fibrin values much above the normal level.

The experiments given in table 15 are complicated by a number of factors but they give a very important lead which is followed out in other experiments. We note in passing that some of these data have been reported elsewhere in connection with experiments dealing with liver necrosis and repair as influenced by dietary and other factors (4), (5). Three different group experiments are included in this table. Each group of experiments was performed upon a litter of pups under approximately identical conditions. The only variable was the special diet for a period of four days preceding the unit chloroform anesthesia of one hour. All pups were killed with ether 48 hours after the chloroform anesthesia. The blood fibrin was determined at autopsy and the extent of liver necrosis and fatty degeneration estimated from histological sections of the liver.

TABLE 13

Individual fluctuations of blood fibrin under standard conditions; dog 15–56; white bull, male; mixed diet

DATE	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
			Average	100 cc. Plasma	100 cc. Blood	
	cc.	*per cent*	*mgm.*	*mgm.*	*mgm.*	
12/21/18	10.1	55	5.1	310	140	
1/ 6/19	9.7	56	4.6	278	130	Weight 47 lbs.
1/13/19	10.4	58	4.9	300	127	
1/20/19	9.2	54	4.7	291	134	
1/24/19	9.0	53	5.5	340	159	
1/27/19	9.3	53	4.7	288	137	
2/ 4/19	8.8	51	4.75	292	143	Weight 46.2 lbs.
Maximum				340	159	
Minimum				278	130	
Variation				62	29	

When a severe liver necrosis is present we expect to find low fibrin values and some of these pups show this reaction but not to an extreme degree. When a slight liver injury is found we may observe a rise above normal in fibrin values. This may be explained in part as a stimulating or irritative reaction similar to the abscess reaction given in later experiments (paper IV). But we note further that the pups fed meat and liver show remarkably high fibrin values as compared with carbohydrate feeding. We believed other factors besides liver injury were concerned and decided to test out various limited diets to ascertain whether or not such diets could influence the level of blood fibrin values in normal dogs. It seemed possible to us that the general stimulating action which protein feeding exerts upon body metabolism might be concerned in the higher level of blood fibrin which may obtain on a meat diet. It is clear that this factor alone will not explain all the reactions noted below, particularly the fact that different proteins of animal origin have very different stimulating effects upon the blood fibrin values. Compare also the low fibrin values with the thyroid feeding (table 22).

TABLE 14

Blood fibrin as influenced by daily bleedings; bled 40 cc. and fed 1 pound rice each day; dog 19–21; adult female pointer

DATE	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
			Average	100 cc. Plasma	100 cc. Blood	
	cc.	*per cent*	*mgm.*	*mgm.*	*mgm.*	
2/10	9.0	51	4.90	334	171	After 3 day fast Weight 37 lbs.
2/19	9.5	49	6.70	394	199	Rice diet for 9 days
2/19	Dog bled 40 cc. per day subsequently					
2/21	9.9	43	6.20	365	207	
2/23	8.6	46	6.85	416	225	
2/26	9.1	42	6.90	410	239	Normal Weight 37 lbs.
2/28	10.1	45	6.20	365	203	Weight 37.7 lbs.
3/ 4	9.5	43	5.80	342	200	Weight 37.7 lbs.
3/ 7	10.6	46	6.35	375	202	Weight 37.3 lbs.
3/11	10.1	45	5.90	347	193	Weight 40 lbs.
3/17	10.0	44	6.45	380	213	Weight 39.2 lbs.
3/19	9.1	46	6.60	397	216	Weight 38.5 lbs.
3/25	9.2	41	5.70	337	198	Weight 38 lbs.
3/29	10.3	44	6.30	336	206	Weight 41 lbs.
4/ 2	10.0	42	5.60	346	201	Weight 42 lbs. Normal Bleeding discontinued
Maximum				416	239	
Minimum				334	171	
Variation				82	58	

TABLE 15

Blood fibrin levels influenced by diet factors plus chloroform injury; four days special diet followed by one hour chloroform anesthesia; pups of 4–10 weeks of age—three different litters

DOG NUMBER	WEIGHT	DIET	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			LIVER		REMARKS
					Average	100 cc. Plasma	100 cc. Blood	Necrotic	Fat	
	lbs.		*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	*per cent*	*per cent*	
3A	5.0	Milk	8.8	49	6.45	395	201	0	20	Skim milk
3C		Carbohydrate	9.3	38	4.85	284	177	0	33	Potatoes
3D		Meat	9.3	46	9.50	569	309	20	66	Cooked skeletal muscle
3E		Fat	9.3	44	2.65	157	89	25	80	Lard, butter and oil
3F	5.6	Liver	9.1	38	17.00	1000	621	0	tr.	Cooked liver
4A	6.4	Gelatin	9.2	52	1.55	95	45	40	tr.	25 grams gelatin (stomach tube)
4B	6.0	Fat	8.8	48	1.50	91	48	80	tr.	Beef fat and crackermeal
4C		Fat and carbohydrate	9.1	39	2.60	150	91	35	20	
4E	5.2	Carbohydrate	9.1	46	5.20	313	169	0	tr.	Potatoes, rice, crackermeal and sugar
4F	3.4	Meat	9.5	38	11.10	657	408	tr.	50	Cooked skeletal muscle
5A	6.2	Gelatin and sugar	9.5	41	2.85	162	96	tr.	50	25 grams gelatin and 25 grams cane sugar
5B	6.0	Beef heart	9.1	43	1.40	83	48	50	20	Cooked beef heart
5C	6.2	Fat and carbohydrate	9.5	48	2.75	162	85	75	20	Butter, lard, potatoes
5D	5.7	Liver	9.2	44	5.45	324	183	0	75	Cooked liver
5E	4.4	Meat	9.5	37	2.85	166	104	50	20	Cooked skeletal muscle

A considerable number of observations have established the fact that for a given dog the fasting value for fibrin is comparatively stable. The fasting value for blood fibrin is established with considerable uniformity by a fasting period of 3 days, regardless of the diet given preceding the fast. We note alternate periods of fasting and limited diet feeding in three parallel experiments (tables 16, 17 and 18). The sequence of diets is the same in all dogs and the duration of the experiments is also identical. It is possible that a change of this sequence might modify the reaction. But it seems pretty clear that the heavy animal protein feeding as a rule gives higher fibrin values. The changes are not great but they are pretty uniform and exceed any fluctuations noted during fasting periods. The very young dog, the adult and the old dog all react much alike.

A remarkable reaction due to an *unusual diet factor* is recorded in tables 19 and 20. Cooked pig stomach fed in liberal amounts seems to be responsible for lifting the blood fibrin level almost 100 per cent above the fasting level. This high level is well sustained (table 19) for a week on this unusual diet and promptly subsides to the normal level when this food factor is withdrawn. The pig stomachs were received fresh, were then washed, cut up and cooked thoroughly in boiling water. The water was poured off and this material was then fed in the amounts indicated. This food was eaten with relish by both dogs.

The second experiment records the prompt reaction which is observed in the blood fibrin level following a large feeding of cooked pig stomach. We are unable to state what factors in this diet may be responsible for this truly remarkable reaction. One should first test the intestinal epithelium and the smooth muscle, but the necessary experiments are not yet at hand. The reaction to liver epithelium may indicate that gland cells are particularly concerned in this peculiar diet reaction.

Pregnancy may be classed as a physiological reaction and it is of interest to record the findings during normal pregnancy in a dog (table 21). The normal level during the last two weeks of gestation is fairly constant in view of the fact that the dog had a very liberal mixed diet. The uniform fall of the hematocrit during this time is of considerable interest but need not be discussed here except to note its importance in explaining the rise in the fibrin values for whole blood. It is quite remarkable that there should be a distinct fall in fibrin values in the two to three days following birth of the pups. There is a noticeable rise above normal in fibrin values on the seventh to tenth days after

TABLE 16

Blood fibrin influenced by diet factors; normal pup 19–2

DATE	WEIGHT	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
				Average	100 cc. Plasma	100 cc. Blood	
	lbs.	*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	
9/13	25.2	9.1	57	5.30	333	143	Analysis after 3 day fast
9/26	23.7	10.3	53	4.60	277	129	Fasted 12 days
9/26	Diet: Rice, crackermeal and sugar, 100 calories per kilo						
9/27		10.0	50	4.70	282	141	
9/28		8.9	57	5.30	358	155	
9/29	23.3	9.2	49	4.85	294	149	
	3 days' fast						
10/1	22.0	9.0	52	4.80	295	141	
10/1	Diet: Lard and butter mixed with crackermeal and weak soup, 100 calories per kilo						
10/2	22.1	9.8	45	4.85	287	159	
10/4	22.0	8.6	51	4.70	291	142	
10/6	22.1	8.7	48	3.70	226	117	
	3 days' fast						
10/8	Diet: Milk and crackermeal, 100 calories per kilo						
10/9	21.0	9.3	54	5.65	348	161	
10/11	21.8	9.5	47	5.70	342	180	
	3 days' fast						
10/17	Diet: Beef heart, 100 calories per kilo						
10/18	20.8	9.4	52	5.30	317	152	
10/21	21.5	9.6	52	6.35	386	185	
	3 days' fast						
10/24	Diet: Cooked pig's or beef liver, 100 calories per kilo						
10/25	21.0	9.6	54	5.05	309	142	
10/26	20.4	9.4	60	7.60	480	194	
10/27	20.6	9.8	57	7.10	448	195	Dog active

TABLE 17

Blood fibrin influenced by diet factors; normal adult dog 19–21

DATE	WEIGHT	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
				Average	100 cc. Plasma	100 cc. Blood	
	lbs.	*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	
9/13	38.6	9.7	54	4.95	302	139	Analysis after 3 day fast
9/26	35.5	10.0	61	5.00	314	122	Fasted 12 days
9/26	Diet: Rice, crackermeal and sugar, 100 calories per kilo						
9/26		10.3	57	7.40	454	194	
9/27		11.1	53	6.90	398	229	
9/29	36.9	9.6	49	7.30	439	224	
	3 days' fast						
10/ 1	35.0	9.4	51	6.75	411	201	
10/ 1	Diet: Lard and butter mixed with crackermeal and weak soup, 100 calories per kilo						
10/ 2	35.5	7.7	51	7.30	330	173	
10/ 3	35.2	10.5	47	5.00	300	157	
10/ 6	35.3	10.6	52	6.20	371	179	
	3 days' fast						
10/ 8	Diet: Milk and crackermeal, 100 calories per kilo						
10/ 9	35.6	9.7	52	6.50	393	191	
10/11	35.8	9.5	46	6.60	394	214	
	3 days' fast						
10/17	Diet: Beef heart, 100 calories per kilo						
10/18	33.2	10.0	49	7.20	430	220	
10/21	33.8	9.6	49	7.00	421	215	
	3 days' fast						
10/24	Diet: Cooked pig's or beef liver, 100 calories per kilo						
10/25	32.3	9.6	47	5.25	314	167	Food not eaten well
10/26	33.0	9.8	50	6.00	361	180	
10/27	32.6	9.5	51	5.85	354	175	

TABLE 18

Blood fibrin influenced by diet factors; normal old dog 19–1

DATE	WEIGHT	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
				Average	100 cc. Plasma	100 cc. Blood	
	lbs.	*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	
9/13	39.0	9.4	46	6.15	368	198	Analysis after 3 day fast
9/26	34.8	10.9	50	6.40	391	196	Fasted 12 days
9/26	Diet: Rice, crackermeal and sugar, 100 calories per kilo						
9/27	36.3	10.3	44	5.90	345	194	
9/28	35.8	9.6	42	6.20	365	213	
9/29	37.3	9.9	45	6.90	408	185	
	3 days' fast						
10/ 1	35.0	9.6	47	7.00	418	222	
10/ 1	Diet: Lard and butter mixed with crackermeal and weak soup, 100 calories per kilo						
10/ 2	35.2	9.0	49	8.00	408	209	
10/ 3	34.9	9.7	48	5.70	342	179	
10/ 6	35.4	10.1	44	8.70	512	285	
	3 days' fast						
10/ 8	Diet: Milk and crackermeal, 100 calories per kilo						
10/ 9	34.8	9.9	46	9.80	604	324	
10/11	35.8	10.3	44	8.55	501	283	
	3 days' fast						
10/17	Diet: Beef heart, 100 calories per kilo						
10/18	33.6	9.9	46	8.80	523	280	
10/21	34.7	9.9	48	8.70	520	268	
	3 days' fast						
10/24	Diet: Cooked pig's or beef liver, 100 calories per kilo						
52/01	34.0	9.7	48	8.65	519	268	
10/26	34.2	9.9	54	8.60	523	243	
10/27	34.0	9.3	49	8.75	530	268	

TABLE 19

Blood fibrin influenced by diet of cooked pig stomach; dog 19-68; spitz, male

DATE	WEIGHT	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
				Average	100 cc. Plasma	100 cc. Blood	
	lbs.	*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	
4/ 3	16.0	10.1	53	5.80	350	166	After 3 day fast
4/ 5	Fed cooked pig stomach—1 lb. daily						
4/ 5	14.6	10.0	49	5.30	308	157	
4/ 7		10.1	47	5.90	350	186	
4/ 8		10.0	46	10.00	592	320	
4/ 9		9.9	44	12.90	762	424	
4/10	15.5	9.2	41	11.50	681	400	
4/11		10.1	38	10.30	612	382	
4/14	15.4	9.5	46	10.60	634	340	Normal
	Feeding of cooked pig stomach discontinued						
4/16		9.3	46	6.60	396	213	
4/18	14.6	9.6	46	5.60	334	183	

TABLE 20

Blood fibin influenced by diet of cooked pig stomach; dog 19–124; mongrel, male, weight 30 lbs.

DATE	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
			Average	100 cc. Plasma	100 cc. Blood	
	cc.	*per cent*	*mgm.*	*mgm.*	*mgm.*	
7/23	10.3	59	5.70	353	144	After 3 day fast
10 a.m.	Fed 2 lbs. cooked pig stomach					
11 a.m.	10.0	57	6.00	370	159	
12 m.	10.8	59	6.35	390	161	
1 p.m.	8.4	57	6.10	390	167	
1 p.m.	Fed 2 lbs. cooked pig stomach					
3 p.m.	10.3	55	8.40	512	228	
4 p.m.	10.6	54	8.60	518	238	
7/24	9.7	58	7.00	435	182	
10 a.m.	Fed 2 lbs. cooked pig stomach					
12 m.	7.6	51	5.85	285	143	
2 p.m.	10.5	49	6.40	379	195	
7/25	6.9	47	8.40	534	285	
7/26	5.2	44	7.00	470	262	
7/28	8.9	54	6.80	423	195	

delivery. It seems best to explain this rise as due in part at least to the reaction at the placental sites. This is a reaction similar in many respects to the reaction taking place about injured tissue anywhere in the body (aseptic abscess, peritonitis, etc.). Such reactions if uncomplicated always cause distinct increase in the blood fibrin values (refer to table 36 below).

TABLE 21

Blood fibrin during pregnancy; dog 18–40; adult mongrel; liberal mixed diet

DATE	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
			Average	100 cc. Plasma	100 cc. Blood	
	cc.	*per cent*	*mgm.*	*mgm.*	*mgm.*	
12/27	9.0		6.60	403		Weight 30.8 lbs.
12/30	9.4	48	5.75	346	181	
1/ 2	8.5	44	6.55	395	223	Weight 31.8 lbs.
1/ 4	9.5	41	6.90	409	240	
1/ 6	10.4	38	7.80	450	277	
1/10	10.1	37	6.60	381	242	
1/13	Pups born in early morning—slight loss of blood					
1/13	10.0	36	4.55	262	168	
1/15	10.2	38	5.20	300	185	Pups nursing. Dog in good condition
1/20	9.3	41	8.30	490	290	
1/22	10.2	40	8.90	517	310	Weight 25.2 lbs.
1/24	10.3	42	8.80	521	303	
1/27	9.5	41	6.50	383	226	
2/ 4	9.5	42	6.60	390	226	
2/10	8.2	41	7.80	478	281	
2/17	8.7	45	8.20	395	208	
2/24	8.9	49	6.15	376	190	Pups normal and nursing
3/ 5	9.0	50	6.40	391	196	Weight 23.2 lbs.
3/11	9.8	53	7.30	444	209	Excellent condition
3/19	10.1	49	6.20	384	160	Nursing pups

Thyroid feeding in small amounts may give a reaction properly called physiological but larger amounts may cause reactions classed as pathological. The amount of thyroid fed in this experiment may be considered large but we wished to give enough of the dried gland to cause loss of weight. With this reaction we feel confident that there was a distinct increase in the daily output of urinary nitrogen, increased endogenous metabolism and consequent breaking down of tissue protein. How will this reaction compare with the inflammatory reaction? In both instances we have a decided increase in tissue katabolism. Inflammation whether septic or aseptic will give a rise in the blood fibrin level but we note no such reaction following thyroid feeding (table 22). There seems to be a gradual lowering of the blood fibrin level as the loss of body weight due to thyroid feeding progresses. This type of tissue katabolism does not call out a reaction on the part of the fibrin similar to that constantly observed in association with the tissue katabolism of inflammation or cell necrosis.

TABLE 22

Blood fibrin influenced by thyroid feeding; dog 19–21

DATE	WEIGHT	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
				Average	100 cc. Plasma	100 cc. Blood	
	lbs.	*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	
7/ 5	37.00	9.8	44	8.10	522	291	
7/ 7	Fed cooked rice—1 lb. daily						
7/ 7		9.9	43	7.60	480	274	
7/ 9		10.4	44	7.70	451	252	
7/10	Fed 1 lb. cooked rice and 1 gm. desiccated thyroid (Armour) daily						
7/10		10.1	39	7.70	448	273	Normal
7/12	35.00	10.1	40	6.00	350	210	
7/14	34.75	9.9	46	7.60	450	245	
7/16		9.5	47	7.40	433	233	
7/18	33.75	9.9	44	6.10	360	203	
7/19	32.00	10.5	45	5.90	346	191	Normal

SUMMARY

Fibrin is a blood plasma protein which is very labile and its fluctuations are due to a variety of stimuli. Many of these stimuli have been recognized.

Our experiments indicate that *diet substances* may be concerned in these fluctuations of fibrin values.

Individual dogs under uniform conditions have a relatively constant fibrin level. Fluctuations should not exceed 20 to 25 per cent.

Fasting dogs have individual blood fibrin levels which are relatively constant. This level is usually established by a 3-day fast.

Normal blood plasma fibrin values should fall within the limits of 250 to 500 mgm. per 100 cc. plasma. The figures for whole blood should always be given as this is the *efficient fibrin level* which concerns hemorrhage. In normal dogs this figure is a little less than one-half the plasma values as the red cell hematocrit is usually 50 to 57 per cent.

Diets rich in animal protein (meat, liver, etc.) favor a high blood fibrin level as contrasted with fasting, fat or carbohydrate feeding.

A striking reaction is noted in association with a diet of cooked pig stomach. This diet causes a rise in the blood fibrin level which may be an increase of 100 per cent over the fasting level.

Pregnancy may show fluctuations in the blood fibrin values which are difficult to explain.

Thyroid feeding sufficient to cause loss of weight may be associated with a slowly decreasing level of blood fibrin.

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Reprinted from The American Journal of Physiology
Vol. 58, No. 3, January, 1922

BLOOD FIBRIN STUDIES

III. Fibrin Values Influenced by Transfusion, Hemorrhage, Plasma Depletion and Blood Pressure Changes

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Received for publication July 19, 1921

At various times in this laboratory the properties of the blood serum proteins have been investigated. The remarkable *stability* and *stabilizing value* of the *blood serum proteins* have been established by these experiments (1). Usually the blood plasma protein (fibrin or fibrinogen) was followed in these same experiments. Also the investigation of many problems related to the circulating blood volume has enabled us to make fibrin determinations in experiments of the type recorded in this paper without duplication of effort. Some of these studies in blood volume have been published (2) and others will follow in the near future.

Fibrin is a very labile substance when compared with the other blood proteins. Sudden changes in blood volume may be brought about by transfusion, hemorrhage or any combination of transfusion and hemorrhage. The blood fibrin concentration is thereby modified. What reaction is to be expected and how does the fibrin reaction compare or contrast with the reaction of the stable serum proteins? Will sudden and extreme changes in blood pressure cause any reaction in the blood fibrin values? The experimental data given below furnish the answer to these questions.

It may be recalled that experiments done in this laboratory (3) give evidence that the level of blood serum proteins is a pretty fixed constant for any given animal. This is in striking constrast to the fibrin (a plasma globulin) which is influenced by diet factors and many other stimuli which have little or no influence upon the serum proteins. Published experiments (4) show how rapidly (24 hours) the blood fibrin is regenerated after its washing out by plasma depletion, but under similar conditions the serum proteins can be brought back to normal only after a time interval of several days. The site of production of fibrinogen may be the liver aided perhaps by a reserve in other tissues (5). The site of production of the serum proteins may also be the liver but the mechanism seems to be very different. It is a simple matter for the body to form large amounts of fibrin and maintain or raise its blood plasma fibrin level. But it is a difficult matter for the body to form or regenerate the other blood serum proteins when these substances are removed or diluted. This may indicate that the fibrin is being constantly used up and manufactured anew in the body The same reasoning may suggest that the serum proteins are not rapidly used in the body economy. That they are essential to the normal environment of the body cells has been amply substantiated (1).

What evidence can be brought forward to indicate a *constant use and consumption of fibrin in the body?* When the blood will not coagulate in contact with tissues because of lack of fibrinogen or other abnormality, we at once observe in man or animal bleeding about the teeth and in some or all parts of the intestinal tract. This indicates that small injuries in the mouth cavity, especially about the teeth, and in the stomach or intestine are constantly present and are sufficient to rupture capillary vessels. Normally these breaks are repaired at once by the use of fibrin as the body of the clot. Probably all types of pressure or slight injury to the surface of the body may break the capillary wall and require fibrin to seal these breaks. We are familiar with the large black and blue areas which follow trivial bruises in patients whose blood does not clot normally in contact with tissues. All types of inflammation and cell injury stimulate fibrin production and probably are associated with a deposition of fibrin and so its withdrawal from the blood plasma. All these facts point to a rapid production and consumption of fibrinogen in the body.

Experimental observations. The greater part of these experiments was done with the primary object of investigating changes in the volume of the circulating blood. Many of these observations have been published (2). As a part of this work blood samples were taken both for fibrin estimation and for blood volume determinations at intervals as stated in the tables. Solutions of inert red dyes (vital red group (2)) were likewise introduced as a part of the blood volume estimation. The influence of these procedures upon the fibrin values is shown in a control experiment in which the usual dye injections and blood sample removals were performed at the usual time intervals *without* any injection, transfusion or hemorrhage. The control experiment (table 27) shows a fibrin level which remains practically unchanged throughout the period of observation. The slight increase after 24 hours is probably due to the bleedings of the preceding day.

All injections were made by hypodermic needle inserted in the jugular vein. No anesthetic was required. Three experiments (tables 30 and 31) were performed under ether anesthesia as cannulae were placed in both artery and vein. This technique has been described elsewhere.

TABLE 23

Intravenous injection of Locke's solution

Dog 18–39. Mongrel. Male. Weight 27 lbs.

DATE	R. B. C. HEMATOCRIT	FIBRIN			PLASMA VOLUME	REMARKS
		Average	100 cc. Plasma	100 cc. Blood		
	per cent	*mgm*	*mgm.*	*mgm.*	*cc.*	
July 11	55	6.7	393	179	479	
Rapid injection into jugular vein—600 cc. Locke's solution						
July 11	45	5.5	315	172	685	Immediately after
July 11	45	5.0	294	161	474	3 hours after
July 12	50	9.9	662	335	493	
July 13	42	8.3	500	291		
Dog 19–99. Bull dog. Male. Weight 33 lbs.						
July 11	36	9.3	600	384	886	
Rapid injection into jugular vein—750 cc. Locke's solution						
July 11	33	8.3	520	351	1195	Immediately after
July 11	31	9.2	574	396	1145	3 hours after
July 12	35	10.8	704	460	790	
July 13	35	8.5	542	361		

Table 23 presents the data from two experiments in which large amounts of Locke's solution were given rapidly intravenously. These experiments are to be compared with subsequent experiments in which acacia solutions or serum are substituted for the Locke's solution. We note a distinct difference. Following the injection of Locke's solution there is a fibrin reaction not observed after the injection of acacia. Therefore the simple dilution of the plasma fibrinogen values is not responsible. It appears more probable that the very presence of these salts (Locke's solution) in unusual concentration in the plasma is responsible for the rise in fibrin values observed after such injections.

Table 23 therefore shows a positive wave of increase of blood fibrin on the day following the injection of Locke's solution. In the second experiment, with an abnormally high fibrin value, the level is further increased on the next day following the injection. This rise is temporary and the return toward normal is evident on the second day following the injection (refer also to table 31). The blood volume figures are given but will not be discussed at this time. These figures indicate a very rapid escape of the Locke's solution from the blood circulation.

TABLE 24

Intravenous injection of acacia solution (6 per cent)

Dog 18–123

DATE	R. B. C. HEMATOCRIT	FIBRIN			PLASMA VOLUME	REMARKS
		Average	100 cc. Plasma	100 cc. Blood		
	per cent	*mgm.*	*mgm.*	*mgm.*	*cc.*	
August 8	53	6.6	480	210	1063	
Intravenous injection—520 cc. of 6 per cent acacia solution						
August 8	39	3.4	219	133	1613	5 minutes after injection
August 8	37	3.8	248	157	1240	3 hours after injection
August 9	43	7.0	459	262	1068	
Dog 17–38						
August 8	58	5.9	456	194	1416	
Intravenous injection—635 cc. of 6 per cent acacia solution						
August 8	41	2.6	168	59		5 minutes after injection
August 8	40	3.2	205	122	1231	3 hours after injection
August 9	45	6.1	398	221	1315	

Table 24 shows two experiments in which acacia solutions 6 per cent were given intravenously in amounts approximately equal to the determined plasma volume. It is known that acacia solutions disappear slowly from the blood stream (6),(7). Therefore the dilution of the fibrin values would be more lasting than following the Locke's solution injections. The fibrin reaction to this dilution is practically nil and we see a slow return to normal which may well parallel the removal of the acacia from the blood plasma. The behavior of the blood volume and hematocrit figures are of great interest but cannot be discussed at this time. It should be kept in mind that each determination means a considerable loss of blood (50 to 60 cc.) as several analyses were made in each period and this explains some of the hematocrit fluctuations.

Table 25 shows again the same general type of reaction noted in the preceding table. Smaller amounts of acacia solutions are injected. These experiments illustrate an interesting reaction which may lead to serious errors in fibrin determination. Blood removed immediately after acacia injection often does not clot and normal coagulation is only obtained by the use of serum. At times this antithrombin reaction will be less definite and may only *delay* blood coagulation, rendering it incomplete for many hours. If this is not controlled, incomplete fibrin values may be returned.

TABLE 25

Intravenous injection of acacia solution (6 per cent)

Dog 19–126. Mongrel. Male. Weight 39 lbs.

DATE	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
		Average	100 cc. Plasma	100 cc. Blood	
	per cent	*mgm.*	*mgm.*	*mgm.*	
April 8	49	10.7	708	363	Blood volume 1867 cc.
Intravenous injection—590 cc. of 6 per cent acacia solution					
April 8	37	5.8	377	216	After injection, coagulation only on adding serum
April 8	43	6.4	407	233	3 hours later
April 9	44	9.5	354	199	
Dog 18–4. Mongrel. Female. Weight 42 lbs.					
April 9	60	7.2	522	208	Blood volume 1962 cc.
Intravenous injection—630 cc. of 6 per cent acacia solution					
April 9	53	3.8	259	121	After injection, coagulation only on adding serum
April 9	52	4.6	319	154	3 hours later
April 10	50	6.6	430	214	

Table 26 also gives data on acacia injection. Smaller amounts of concentrated (20 per cent) acacia solution were injected. One experiment shows an absolutely negative reaction although the initial value for blood fibrin is abnormally high. The second experiment shows immediate fluctuation and a slight increase on the second day.

Table 27 is of considerable interest as it gives control of all the factors concerned in the preceding experiments—that is, dye injection and blood sample removal. The procedure is identical in every respect but for the fact that no injection of acacia or Locke's solution is made. There are slight fluctuations during the period of observation but the fibrin values are relatively constant. The slight rise on the second day is to be explained by the hemorrhages of the first day due to removal of blood samples.

TABLE 26

Intravenous injection of concentrated acacia solution (20 per cent)

Dog 18–24. Mongrel. Weight 30 lbs.

DATE	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
		Average	100 cc. Plasma	100 cc. Blood	
	per cent	*mgm.*	*mgm.*	*mgm.*	
March 12	61	10.95	815	315	Blood volume 1439 cc.
Intravenous injection—55 cc. of 20 per cent acacia solution					
March 12	53	9.30	643	299	20 minutes after injection
March 12	51	9.10	613	298	3 hours later
March 13	55	9.70	675	304	
March 17	51	7.75	608	297	
Dog 17–160. Mongrel. Male. Weight 48.5 lbs.					
March 21	54	7.40	549	251	Blood volume 2166 cc.
Intravenous injection—110 cc. of 20 per cent acacia solution					
March 21	55	5.90	393	208	After injection
March 21	56	6.20	433	212	4 hours later
March 21	47	5.90	390	209	7 hours later
March 22	50	10.20	673	339	

Table 28 shows the negative results which may follow the introduction of fresh normal dog serum into the circulation of normal dogs. Both dogs show an abnormally high fibrin level and may have had a mild distemper infection. The drop in fibrin values is not quite the expected amount considering the amount of serum injection. The high fibrin values are not again attained on the day after the experiment and the red blood cell hematocrit still remains low. We have reason to believe that these hematocrit figures of the second day are to be explained by the removal of many blood samples during the experiment, rather than by a residue of serum remaining for 24 hours increasing the plasma volume and giving a relative decrease in red cells.

TABLE 27

Control experiment. Samples removed but no injection
Dog 18–116. Bull. Female. Weight 39 lbs.

DATE	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
		Average	100 cc. Plasma	100 cc. Blood	
	per cent	*mgm.*	*mgm.*	*mgm*	
March 25	56	6.65	462	201	Blood volume 1867 cc.
March 25	57	7.00	484	210	"After"
March 25	50	6.20	415	206	3.5 hours later
March 26	50	7.60	515	257	

TABLE 28

Intravenous injection of normal serum
Dog 19–114. Fox terrier. Female. Weight 18.8 lbs.

DATE	R. B. C HEMATOCRIT	FIBRIN			REMARKS
		Average	100 cc. Plasma	100 cc. Blood	
	per cent	*mgm.*	*mgm.*	*mgm.*	
March 11	57	11.7	841	361	Blood volume 892 cc.
Intravenous injection—290 cc. normal dog serum					
March 11	45	7.5	513	283	10 minutes after
March 11	47	7.2	473	250	1.3 hours after
March 11	39	8.7	562	388	4 hours after
March 12	44	11.4	717	399	
Dog 18–92. Fox terrier. Male. Weight 20 lbs.					
March 19	53	13.1	898	422	Blood volume 1041 cc.
Intravenous injection—400 cc. normal dog serum					
March 19	47	8.6	546	289	15 minutes later
March 19	43	7.9	508	290	3 hours later
March 20	41	8.9	582	341	
March 22	41	8.7	558	328	

Table 29 presents some interesting fluctuations in blood fibrin values. We are not prepared to explain the fall in fibrin values which is noted in both experiments 1.5 hours after the injection of whole blood. We may assume that this induced plethora must be adjusted in some fashion by the body, that all the blood proteins are removed from the circulation to some extent and the fibrin takes its part in this passive reaction. The normal fibrin level is reached 24 hours later.

TABLE 29

Intravenous injection of whole blood

Dog 18–38. Coach. Female. Weight 24 lbs.

DATE	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
		Average	100 cc. Plasma	100 cc. Blood	
	per cent	*mgm.*	*mgm.*	*mgm.*	
February 28	48	10.3	688	355	Blood volume 1197 cc.
Intravenous injection—400 cc. whole blood—donor's blood=55 per cent r.b.c.					
February 28	51	8.4	564	277	After injection
February 28	50	5.0	340	170	1.5 hours later
March 1	49	9.9	660	336	
March 3	51	9.2	616	304	
Dog 621. Mongrel. Female. Weight 41 lbs.					
March 5	32	6.6	421	285	Blood volume 1614 cc.
Intravenous injection—700 cc. whole blood					
March 5	44	7.0	478	270	After injection
March 5	43	5.1	341	194	1.3 hours later
March 5	39	5.2	343	208	4 hours later
March 6	41	6.9	454	266	
Donor	38	8.5	563	352	350 cc. blood given
Donor	49	7.0	485	246	350 cc. blood given

The blood infusion was done by means of hypodermic needles placed in the veins of both donor and recipient and a well vaselined syringe to transfer and measure the whole blood. There was no reaction following this procedure. In the second experiment the blood fibrin and red cell hematocrit values of the donor's blood are recorded.

Table 30 records two experiments which have been described in part elsewhere (8). It is not necessary to give in detail the experimental method. This exchange reduces the fibrin level without any complicating shock or reduction of blood serum proteins. As the dog is bled from an artery a similar amount of blood serum and red corpuscles are injected intravenously. A period of 10 to 15 minutes suffices for this procedure which washes out much of the circulating fibrinogen. The regeneration of fibrinogen is prompt and obvious in 3 hours after the exchange. The normal level is usually attained or even exceeded by the end of 24 hours.

TABLE 30

Fibrin depletion—Bled from artery and transferred simultaneously with a mixture of serum plus corpuscles

Dog 19–74. Terrier. Female. Weight 16 lbs.

DATE	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
		Average	100 cc. Plasma	100 cc. Blood	
	per cent	*mgm.*	*mgm.*	*mgm.*	
August 2	58	8.3	600	255	
Exchange = 144 per cent total blood volume					
August 2	49	1.5	75	38	End of exchange
August 2	50	4.4	304	152	3 hours later
August 3	45	8.4	499	281	
August 4	41	11.8	690	411	Infected neck wound
August 5	40	15.0	878	526	Infected neck wound
August 14	25	11.1	636	476	Clean granulation
Dog. Young pup. Weight 15 lbs.					
August 5	42	6.8	435	253	
Exchange = 176 per cent total blood volume					
August 5	45	4.2	305	161	3 hours after exchange
August 6	42	9.9	650	378	
August 7	27	8.4	484	356	Condition good

We note a considerable rise in blood fibrin in the first experiment (table 30) on the third and fourth days following the exchange. This is to be explained by the presence of an infected neck wound. Much more data on infection will be presented in the next paper.

Table 31 cites an experiment which has been described in detail elsewhere (4). A large number of similar experiments has been summarized and we need only state that the fibrin depletion is considerable and that its repair or regeneration is complete within 24 hours. The serum protein regeneration on the contrary is slow and not completed usually for several days. This experiment is a good example of a large group. The rise of the fibrin level above normal may be explained in part by slight infection at the operative site.

TABLE 31

Plasma depletion—Bled from artery and infused Locke's solution simultaneously, 800 cc. each

Dog 19-124. Mongrel. Male. Weight 36 lbs.

DATE	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
		Average	100 cc. Plasma	100 cc. Blood	
	per cent	*mgm.*	*mgm.*	*mgm.*	
May 5	50	6.70	457	228	
Bled 800 cc. and infused Locke's solution 800 cc.					
May 5	33	3.40	216	146	3 hours after exchange
May 6	27	7.10	444	323	
May 7	28	9.70	554	400	
May 8	31	10.40	594	403	
May 9	31	11.20	643	405	
May 11	31	11.75	672	464	
May 14	30	10.30	588	412	

TABLE 32

Hemorrhage followed by infusion; bled 725 cc. followed by intravenous injection of Locke's solution, 725 cc.

Dog 19-111. Weight 39.5 lbs.

DATE	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
		Average	100 cc. Plasma	100 cc. Blood	
	per cent	*mgm.*	*mgm.*	*mgm.*	
June 17	46	7.0	402	219	Before bleeding
Bled 725 cc.—Fall in B.P. from 158 to 40 mm. mercury					
June 17	39	5.6	315	193	After bleeding
Intravenous injection of Locke's solution—725 cc.					
June 17	24	4.1	227	172	After injection
June 17	31	5.0	281	194	3 hours later

Table 32 gives the reaction associated with a large hemorrhage and definite shock followed by a saline transfusion. The fibrin level and

TABLE 33

Hemorrhage without infusion

Dog 17–160. Mongrel. Weight 46 lbs.

DATE	R. B. C HEMATOCRIT	FIBRIN			REMARKS
		Average	100 cc. Plasma	100 cc. Blood	
	per cent	*mgm.*	*mgm.*	*mgm.*	
May 14	55	9.1	644	289	Blood vol. est. 2090 cc.
Bled 530 cc. from jugular					
May 14	52	8.1	558	268	At end of bleeding
May 14	43	6.6	386	220	3 hours after bleeding
May 15	42	8.2	532	307	
Bled 550 cc. from jugular					
May 15	38	6.0	382	236	At end of bleeding
May 15	34	7.4	485	320	3 hours after bleeding
May 16	31	8.8	567	391	
May 19	29	9.2	578	413	
May 20	35	10.8	705	462	
May 21	32	8.0	517	331	

TABLE 34

Sodium nitrite given intravenously

Dog 19–127. Terrier. Male. Weight 29 lbs.

DATE	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
		Average	100 cc. Plasma	100 cc. Blood	
	per cent	*mgm.*	*mgm.*	*mgm.*	
April 30	48	6.6	470	246	
Sodium nitrite—2 cc. of 2 per cent solution in 20 cc. saline					
April 30	44	7.5	517	290	30 minutes later
April 30	41	6.1	499	265	3 hours later
Dog 19–132. Mongrel. Weight 29 lbs.					
May 6	38	6.8	434	269	
Sodium nitrite—2 cc. of 2 per cent solution in 20 cc. saline					
May 6	39	6.8	455	231	30 minutes later
May 6	41	6.6	425	251	4.5 hours later

hematocrit value after the hemorrhage show that fluid has been thrown into the circulation from the tissues. Further dilution of the fibrin content is produced by the saline infusion. The fibrin value shows a rise in the next three hours and would have returned to normal within 24 hours.

TABLE 35

Adrenalin given intravenously

Dog 19–121. Terrier. Male. Weight 30 lbs.

DATE	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
		Average	100 cc. Plasma	100 cc. Blood	
	per cent	*mgm.*	*mgm.*	*mgm.*	
April 22	35	9.0	579	376	Blood volume 1225 cc.
Adrenalin 4 cc. of 1–1000 solution in 25 cc. saline					
April 22	40	8.7	571	342	30 minutes after injection
April 22	38	9.0	584	359	3.5 hours after injection
Dog 19–99. Bull. Male. Weight 36 lbs.					
April 23	48	7.4	452	237	Blood volume 1487 cc.
Adrenalin 4 cc. of 1–1000 solution in 30 cc. saline					
April 23	51	7.7	533	262	30 minutes after injection
April 23	46	7.1	469	252	3 hours after injection
Dog 19–111. Weight 44 lbs.					
April 19	54	9.3	634	293	Blood volume 1103 cc.
Adrenalin 4.5 cc. of 1–1000 solution in 70 cc. saline					
April 19	55	8.4	575	286	After injection
April 19	55	8.0	556	251	3 hours after injection
Dog 17–160. Mongrel. Weight 46 lbs.					
May 19	29	9.2	591	422	
Adrenalin 2 cc. of 1–1000 solution					
May 19	29	9.4	594	421	After injection
May 19	27	9.4	596	433	3 hours after injection

Table 33 shows the reaction following simple hemorrhage. The dog was bled one-fourth the estimated blood volume on each of two successive days. The fibrin value is high and shows a fall during the period of bleeding and during the next 3 hours. This is probably to be explained by the spontaneous dilution of the blood plasma by fluids contributed from the body tissues. After the second bleeding the sharp fibrin regeneration masks the plasma dilution. The normal level is again established on the day after the second bleeding. It may be pointed out that the *whole blood fibrin values actually rise* during this period of bleeding. The effective fibrin values per 100 cc. whole blood are increased. This represents the ability of whole blood to form a clot and the toughness of the clot depends upon this figure and not on the plasma fibrin values.

It may be claimed that the changes in the fibrin values noted in many of the above experiments are to be explained by great fluctuations in blood pressure. It has been claimed that increase of blood pressure (exercise, adrenalin) may cause some of the blood plasma fluids to escape and thus explain the observed blood concentration. The experiments given here show that such is not the case. Lowering the blood pressure by sodium nitrite or raising the pressure by adrenalin will not show any greater fluctuations in blood fibrin values than are recorded in control experiments (table 27).

The adrenalin experiments usually show a slight amount of red cell hematocrit increase but the fibrin values are constant. These experiments serve as further control of the experimental procedure.

SUMMARY

Plasma fibrin values may be modified by large intravenous injections of Locke's solution. Subsequently there is a rise above the normal level which may be best explained by the stimulating action of the salts.

Acacia injections will produce similar dilution of the fibrin values depending upon the amounts of solution introduced. This is followed by a slow rise of the fibrin values to normal. There is no over-stimulation of fibrin production.

Control experiments show minimal fluctuations in the fibrin values.

Normal serum given intravenously will cause a reaction much like that observed in the acacia experiments.

Induced plethora by means of whole blood transfusion indicates a removal of blood fibrin during the two hours following the injection. This may be a part of the reaction which adjusts the blood volume to its normal extent under such conditions.

Fibrin may be washed out of the circulation by simultaneous bleeding and intravenous injection of a red cell serum mixture. There is a prompt return to normal fibrin values within 24 hours.

Hemorrhage with or without saline transfusion will cause wide fluctuations in fibrin values but the normal level is usually established or exceeded within 24 hours.

Great changes in blood pressure do not by themselves modify the amount of fibrinogen present in the circulating plasma.

All this evidence speaks for a constant manufacture and utilization of fibrin or fibrinogen in the body. This labile blood plasma protein is entirely different in these respects from the other blood plasma proteins.

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Reprinted from The American Journal of Physiology
Vol. 58, No. 3, January, 1922

BLOOD FIBRIN STUDIES

IV. Fibrin Values Influenced by Cell Injury, Inflammation, Intoxication, Liver Injury and the Eck Fistula

NOTES CONCERNING THE ORIGIN OF FIBRINOGEN IN THE BODY

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Received for publication July 19, 1921

All our experiments in this and other papers emphasize the fact that the blood plasma fibrinogen is a very labile substance which reacts to a multitude of stimuli within the body. It has long been recognized that acute inflammations (pneumonia, peritonitis) were invariably associated with high fibrin or fibrinogen values. It was also recognized that liver injuries were very often associated with low fibrin values, especially when the liver was suffering from extensive acute injuries (chloroform, phosphorus). Many investigators have pointed out normal fluctuations in fibrin values but we believe very many factors were not controlled in these earlier observations. Given a constant diet or fasting and normal health, the dog shows small individual fluctuations (20 to 25 per cent) and in time we may hope to understand even these minor changes in fibrin levels. We have been able to show that certain diet factors may raise or depress the normal fasting fibrinogen level (paper II). The active substances in these foods are not known and more work is needed here.

We have given reasons (paper III) why the blood fibrinogen is being used up rapidly in the daily routine of the body. It is obvious in these experiments how promptly a deficit in fibrin values can be made up in a few hours; this in striking contrast to the other blood plasma proteins (albumins, globulins) which are regenerated only with considerable difficulty (6).

Papers by Whipple (8) and Goodpasture (5) supplement each other and furnish evidence that fibrinogen is produced mainly in the liver but suggest that the intestinal tract may supply a certain amount of reserve fibrinogen in certain emergencies. The experiments given below furnish additional evidence to explain the complex problem concerning the use and production of fibrinogen in the body. The evidence is very strong that the *liver* is the main and perhaps only source of supply of fibrinogen. It may be well to note the evidence that a certain reserve of fibrinogen is held in the body, perhaps in the intestinal tract epithelium and elsewhere.

The familiar fact that fibrin levels are very high in pneumonia septicemia and peritonitis has been explained as due to the presence of pathogenic organisms in the blood stream and tissues. Our experiments below make it clear that *dead tissue* and a non-specific (aseptic) inflammation are the only factors needed to give this familiar high fibrinogen level in the blood. It is very probable that aseptic or septic inflammation is the cause of extensive deposit or precipitation of fibrin in the disturbed tissues and this brings about a rapid production with an abnormally high fibrinogen level. The fibrin in the lungs of pneumonia is very conspicuous but we have good evidence that much fibrin is deposited within any area of inflammation. This fibrin is rapidly digested by various ferments and is probably continuously formed in the abnormal area, at least during the acute period of inflammation.

EXPERIMENTAL OBSERVATIONS. The various methods and experimental technique have been fully described. Autopsy protocols with a single exception are not furnished as few of the experiments are fatal. Reference will be made in certain experiments to autopsy records given in other papers (7). Unless note is made to the contrary, the dogs are on a mixed diet consisting of cooked meat, bones, bread, rice, vegetables and so forth.

Sterile abscess. A sterile subcutaneous abscess may be produced promptly in a normal dog by the injection of 1 cc. of spirits of turpentine below the skin. A soft, fluctuant abscess, containing sterile pus, forms in the first 24 to 36 hours. It usually measures about 7 to 10 cm. in diameter and will often rupture spontaneously on the third to fifth day. The reaction following this experimental procedure is strikingly uniform in dogs. There is a prompt rise of the blood fibrinogen level as shown in tables 36 and 37. The maximum fibrin values are usually attained on the second or third day and persist until the abscess ruptures or is drained by surgical incision (refer to table 52). If drainage is free, we note a prompt and progressive fall to normal (table 36) but with poor drainage the inflammation does not subside and the fibrin level remains high (table 37).

These experiments and many other similar ones give proof that *tissue injury* and consequent inflammation are alone necessary for this rise in the blood fibrin values. Other experiments indicate that *tissue injury* unaccompanied by the usual inflammatory reaction is sufficient to give this specific physiological reaction—an over-production of fibrin in the body (table 38).

Toxic proteoses. A few experiments with toxic proteoses are given in table 38. The toxic proteose used was obtained from obstructed intestines and its preparation has been described elsewhere (10). The Witte's peptone was obtained in Germany in 1913. These experiments show several interesting points. The first experiment shows a rise in fibrin values and a small drop in red cell hematocrit. The intoxication was moderate and recovery complete in 24 hours. We know that there is an injury of body protein as there is a great increase in urinary nitrogen (9), but there is no familiar inflammatory reaction on the part of any body tissues. This is significant in emphasizing the importance

TABLE 36

Sterile abscess—turpentine—mixed diet
Dog 19–2. Bull. 25 lbs.

DATE	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
			Average	100 cc. Plasma	100 cc. Blood	
	cc.	*per cent*	*mgm.*	*mgm.*	*mgm.*	
December 30	9.1	45	5.45	327	179	
Turpentine—1 cc. subcutaneously						
December 31	9.6	48	8.00	480	248	Dog active. Abscess forming
January 1	10.0	49	16.40	978	499	Abscess larger and firmer
January 2	9.6	47	14.95	900	474	Abscess 12 × 7 × 4 cm. Fluctuant. Dog slightly toxic
January 3	9.6	42	15.70	926	536	Abscess ruptured. Drainage good
January 4	9.6	42	13.65	805	436	Dog active. Drainage good
January 6	9.2	40	8.80	520	311	
January 8	9.7	42	8.20	483	279	Abscess closed
January 9	9.2	42	8.00	473	279	Dog active
January 11	9.2	45	6.60	394	191	Dog well. Weight 24 lbs.

of *tissue injury* as contrasted with the usual inflammatory reaction. Because the inflammation is conspicuous and much studied its importance as regards the rest of the body has often been overestimated. The *injured tissue* and its disintegration products evidently must be kept in mind and wherever possible viewed apart from the familiar inflammation which usually accompanies any extensive tissue injury.

TABLE 37

Sterile abscess—turpentine—mixed diet
Dog 19–21. Pointer, female. 36 lbs.

DATE	WEIGHT	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
			Average	100 cc. Plasma	100 cc Blood	
December 16	Turpentine—1.2 cc. subcutaneously					
	lbs.	*per cent*	*mgm.*	*mgm.*	*mgm.*	
December 17	36.1	56	8.80	540	238	Abscess forming. Dog toxic
December 18	34.0	54	13.70	852	389	Abscess softer
December 19	33.9	45	16.40	964	530	Abscess broken
December 20	34.8	47	14.40	870	465	Poor drainage
December 21	35.3	46	16.00	945	515	Good drainage. Dog better
December 22		50	14.70	879	444	Poor drainage
December 23	38.1	44	11.50	679	378	Abscess opened again. Dog active
December 24	37.6	39	9.00	526	324	Abscess granulating
December 25	35.8	39	7.65	446	273	Dog well
December 26	36.9	40	8.60	507	306	Abscess healing
December 27	36.9	39	8.10	468	287	Wound clean
December 28	37.5	38	7.35	446	276	Dog active
December 30	36.0	40	6.40	364	217	Dog normal
January 3	35.3	45	5.80	344	188	Dog normal
January 6	35.9	48	5.55	331	171	
January 10	35.5	53	5.40	328	156	
January 13	34.5	44	5.70	339	190	
January 20	34.0	55	5.50	349	158	Blood normal

This establishes normal level for this dog.

The last two experiments (table 38) show the effects of a lethal dose. The post-mortem findings with the remarkable engorgement of the portal area and especially the intestinal mucosa has been described and pictured (11). We note a loss of fluid to the tissues and a rise in the red cell hematocrit (shock). Under such conditions, we believe there is a loss of fibrin in two directions: *1*, a passive escape with the other blood plasma elements; 2, an active escape due to the precipitation of fibrin in certain areas sufficiently injured by the proteoses. The high initial level in the last experiment is due to a distemper infection. The reaction, however, is the same following injection of the Witte's peptone (a mixture of proteoses and peptones). It was necessary to coagulate the plasma by fresh serum.

TABLE 38

Toxic proteose intravenously—sublethal and lethal doses

Dog 19-131. Male. 30 lbs.

TIME	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
		Average	100 cc. Plasma	100 cc. Blood	
	per cent	*mgm.*	*mgm.*	*mgm.*	
	44	6.60	440	246	
10 a.m.	Toxic proteose—intravenous injection—intoxication moderate				
11 a.m.	44	7.90	525	296	Dog toxic, vomited at times
12 m.	42	6.60	445	257	Clinical improvement
5 p.m.	40	7.95	536	322	Dog recovering
Dog 19-93. Male. 17 lbs.					
11 a.m.	41	6.60	462	271	
11 a.m.	Toxic proteose—intravenous injection—lethal intoxication				
12 m.	36	3.80	286	182	Vomiting and diarrhea
12:30 p.m.	47	5.00	336	178	Death. Typical autopsy findings
Dog. Black terrier. 10 lbs.					
	43	9.40	616	352	Distemper
Witte's Peptone, 3 grams—intravenous injection—lethal intoxication					
	45	8.60	560	311	17 minutes after Death in 4 hours

Roentgen ray injury. These experiments are representative of many similar ones performed in this laboratory to study the systemic intoxication due to the x-ray. These results are to be published elsewhere (7). The x-ray experiments are significant as regards the *intestinal* *tract* which has been considered by many as the source of fibrinogen. Others have considered the intestine as contributing to the fabrication of fibrinogen in the liver or as a reserve depot for fibrinogen. Warren and Whipple have many experiments in which large doses of the x-rays given over the abdomen have caused almost complete destruction of the epithelium covering the villi and lining the crypts of the small intestine. These experiments show almost uniform fatal intoxication on the fourth day (table 39) with a *rising curve* of fibrin values. If the intestinal tract were essential for fibrin production, we should expect a prompt fall in blood fibrin similar to that observed uniformly after acute liver injury and extensive necrosis (chloroform).

TABLE 39

X-ray exposures—fatal and non-fatal

Dog. 19–78. Airedale, female. 30 lbs.

DATE	WEIGHT	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
				Average	100 cc. Plasma	100 cc. Blood	
	lbs.	*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	
March 18	27.2	10.1	66	5.9	384	129	
March 18	X-ray exposure over abdomen—350 milliampere minutes						
March 19	26.7	10.1	60	7.0	436	174	Vomiting
March 20	26.4	10.2	61	10.1	631	247	Vomiting and diarrhea
March 21	25.9	9.9	60	15.0	939	375	Very toxic
March 22	24.9	12.6	66	16.1	994	340	Killed with ether

Dog 19–101. Airedale

DATE	WEIGHT	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN Average	FIBRIN 100 cc. Plasma	FIBRIN 100 cc. Blood	REMARKS
February 21	30.3	7.1	62	6.8	460	175	
February 21	X-ray exposure over thorax—350 milliampere minutes						
February 22	30.0	6.3	60	8.4	590	234	Normal
February 23	30.2	5.2	55	7.0	499	226	
February 24	29.1	3.9	59	8.2	667	273	Normal
February 25	28.6	11.2	57	7.4	450	185	
February 26	28.1	9.3	55	6.4	400	172	
February 27	28.2	8.0	60	5.1	325	146	
February 28	27.8	10.8	54	6.4	394	157	

The second experiment, too, is of considerable interest as the x-rays were given over the *thorax* alone. When exposures of this type are used, we can demonstrate a definite cell injury in the lymphatic tissues and bone marrow of ribs and vertebrae. There is no acute inflammatory reaction of the usual type and no obvious deposit of fibrin, yet we note a prompt and considerable rise in blood fibrin values. This observation emphasizes again the non-specific type of this stimulus and further the importance of *cell necrosis* rather than any inflammatory reaction. Probably any *cell injury* in the body, with or without any inflammatory reaction, will cause this characteristic reaction with the prompt rise in blood fibrinogen values.

Acute infection. Distemper in dogs is an acute infection which is much like influenza in human beings. Fatal cases usually show pneumonia, but many cases may show normal lungs (dog 18-108, table 40). We note that the fibrin values are usually high—in fact some of our highest figures in dogs are noted in this acute infection (table 40). Also refer to table 9, paper 1, and table 53, below.

TABLE 40

Severe distemper and intoxication

Dog. 19–33. Fox terrier, female. 18 lbs.

DATE	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
			Average	100 cc. Plasma	100 cc. Blood	
	cc.	*per cent*	*mgm.*	*mgm.*	*mgm.*	
September 2	9.4	42	12.2	719	427	Eyes and nose discharging
September 3	9.6	46	13.1	778	422	Weight 16 lbs.
September 4	9.7	43	13.7	809	459	Dog toxic and dull
September 5	9.8	45	16.8	992	547	Death next day
Dog 18–108. Mongrel, female						
		56	25.0	1570	864	Killed with ether Acute distemper without pneumonia

Chloroform injury of the liver. The three tables (nos. 41, 42 and 43) show four experiments—two stimulating doses and two depressing toxic doses, all non-fatal. We followed the general technique established by Davis and Whipple (2) in their chloroform experiments and wish to use the facts established by them in the general argument. The fasting period preceding the chloroform anesthesia makes for uniformity in extent of the liver injury. The first experiment (table 41) with 45 minutes chloroform anesthesia without doubt caused a certain amount of liver necrosis but this injury was not sufficient to cause a fall in fibrin values. This experiment demonstrates again the fact that *tissue injury* or *necrosis* will call out the fibrin reaction and we note a rise during the seven days following the injury. It is probable that hyaline necrosis of liver cells or other body cells is associated with the deposition or utilization of a certain amount of blood fibrin.

TABLE 41

Chloroform anesthesia—stimulating dose
Dog 19-68. "Chow" dog. 13.7 lbs.

DATE	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
			Average	100 cc. Plasma	100 cc. Blood	
	cc.	*per cent*	*mgm.*	*mgm.*	*mgm.*	
January 8	11.0	62	4.9	309	117	3 day fast
January 8	45 minutes chloroform anesthesia					
January 9	10.9	62	5.6	356	135	Fasting
January 10	10.9	62	6.2	390	148	Fasting
January 11	11.0	62	6.0	378	144	Fasting
January 12	11.5	65	8.4	533	188	Fasting
January 13	11.3	59	8.7	478	198	Fasting
January 14	10.9	54	6.7	407	189	Fasting
January 15	10.9	54	7.6	451	210	Crackermeal and rice diet
January 16	11.0	53	5.2	305	143	Crackermeal and rice diet
January 17	10.0	52	6.0	369	176	Mixed diet
January 18	8.4	46	5.5	343	185	Active
January 19	10.4	44	5.2	309	174	
January 20	9.8	45	5.1	308	168	
January 21	10.0	44	5.3	315	175	

Table 42 shows the results of two experiments with one week's interval between the chloroform anesthesias. The first experiment shows that this old dog was more resistant to 60 minutes chloroform than the average dog (compare table 43). It is known that young animals are more susceptible than adults to chloroform injury. The reaction to the first chloroform is a rise in fibrin values (table 42) similar to that observed in table 41. The second chloroform anesthesia of 75 minutes was given after 7 days when it is probable that the greater part of the primary injury had been repaired (3). We note a distinct fall in fibrin values which is in effect for 4 to 5 days. After that the return to normal is steady and this fibrin curve corresponds in remarkable fashion to the picture of liver cell repair and regeneration which is so clearly understood under these experimental conditions (3). It is to be noted that the curve of fibrin regeneration does not shoot far above normal as observed by Whipple and Hurwitz (12). It is probable that this rise above normal was due to the hemorrhage factor introduced by Whipple and Hurwitz because of the large amounts of blood required for analysis.

TABLE 42

Chloroform anesthesia—stimulating and depressing doses
Dog 19-1. Old bull dog. 38 lbs.

DATE	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
			Average	100 cc. Plasma	100 cc. Blood	
	cc.	*per cent*	*mgm.*	*mgm.*	*mgm.*	
December 11	9.3	47	8.2	490	258	3 day fast
December 11	60 minutes chloroform anesthesia					
December 12	9.5	49	8.8	533	270	Fasting
December 13	10.0	47	8.9	553	291	Fasting
December 15	10.8	45	10.1	608	333	Fasting
December 18	9.8	49	11.7	702	359	Fasting
December 18	75 minutes chloroform anesthesia					
December 19	11.2	45	12.0	704	390	Mixed diet
December 20	11.1	48	4.5	267	140	Cholemia
December 21	11.1	42	4.5	211	122	Cholemia
December 22	11.1	44	4.5	264	152	
December 23	10.9	38	4.7	270	167	
December 24	10.7	40	4.8	269	160	Slight cholemia
December 25	9.0	40	5.1	305	183	
December 26	10.8	38	5.7	331	206	Normal
December 27	9.7	38	6.7	406	252	
December 28	10.4	38	7.8	455	281	
December 30	10.1	43	7.9	470	269	
January 2	11.0	44	8.8	518	290	
January 4	10.6	41	7.9	462	271	
January 6	10.4	44	8.1	481	272	

The last experiment with chloroform (table 43) gives a prompt fall in fibrin values. The lowest figure in such experiments is usually found on the third day, after which the liver repair is active and reflects its activity in the curve of fibrin regeneration. Cholemia is usually noted whenever the liver necrosis is of any considerable extent. Observations continued for two months on this dog showed a uniform level of blood fibrin between 280 to 320 mgm. per 100 cc. plasma. Also refer to table 10 in paper 1 for low fibrin values in more severe chloroform poisoning of the same type. We may refer also to the chloroform injury in the series of pups given in table 15, paper II.

Phosphorus and hydrazine injury of the liver. The two tables (nos. 44 and 45) give sample experiments with phosphorus poisoning. The first experiment (table 44) gives a stimulating dose which did not cause any clinical symptoms of intoxication or loss of appetite. There was no cholemia. On the second, third and fourth days after the administration of phosphorus subcutaneously, we observe considerable increases in the blood fibrin values. The return to normal is complete in about 1 week which corresponds to the time of complete repair of the injury in the liver. The gradual fall in the hematocrit is not due to the removal of the small blood samples but in large part to the liver disturbance. This accounts for the rise in the fibrin values for the *whole blood.* The second experiment (table 45) shows the reaction following a large dose of phosphorus. This amount (1 mgm. phosphorus per pound body weight) is very close to the lethal dose. We observe a fall in fibrin

TABLE 43

Chloroform anesthesia—liver necrosis and low fibrin

Dog 19–66. Fox terrier. 20 lbs.

DATE	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
			Average	100 cc. Plasma	100 cc. Blood	
	cc.	*per cent*	*mgm.*	*mgm.*	*mgm.*	
December 12	10.8	56	5.6	338	151	3 day fast
December 12	60 minutes chloroform anesthesia					
December 13	9.9	59	4.0	250	101	Fasting
December 14	10.0	64	3.1	181	65	Fasting
December 15	10.8	65	2.6	164	58	Cholemia
December 17	10.2	58	3.5	243	105	Cholemia
December 18	10.4	57	4.3	258	112	Fasting
December 19	9.7	61	5.4	345	132	Fasting
December 20	9.8	54	5.1	311	143	Crackermeal and rice diet
December 22	10.0	52	3.4	314	150	Crackermeal and rice diet. Dog active

Observations continued for two months show uniform fibrin level.

TABLE 44

Phosphorus poisoning—stimulating dose
Dog 19–1. Bull, male

DATE	WEIGHT	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
				Average	100 cc. Plasma	100 cc. Blood	
	lbs.	*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	
March 25	39.0	10.3	45	6.8	400	222	Mixed diet
March 25	Phosphorus in oil—31 mgm.—subcutaneously						
March 26	39.0	10.3	45	8.2	482	261	
March 27	40.3	10.2	45	10.0	589	323	Dog lively
March 28	40.5	9.9	40	9.9	580	346	
March 29	41.2	10.0	44	9.5	559	313	
March 31	38.6	10.5	43	7.6	443	253	
April 1	41.2	10.0	42	7.7	451	262	
April 2	40.0	10.2	37	6.9	400	251	
April 3	39.0	10.9	37	6.8	389	249	Dog normal

TABLE 45

Phosphorus poisoning—depressing or toxic dose
Dog 19–123. Mongrel, male

DATE	WEIGHT	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
				Average	100 cc. Plasma	100 cc. Blood	
	lbs.	*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	
April 29	30.0	8.5	43	7.2	435	243	Mixed diet
April 29	Phosphorus in oil—30 mgm.—subcutaneously						
April 30	30.2	9.8	43	9.2	542	310	
May 1	30.1	9.7	38	8.7	507	314	Dog quiet
May 2	30.1	9.6	42	4.3	253	147	
May 3	30.0	9.8	38	2.2	128	79	Cholemia—trace
May 5	29.6	10.3	40	3.0	175	105	Cholemia—marked
May 6	29.7	9.5	37	3.3	191	121	
May 7	29.7	9.4	37	4.3	251	158	Dog active
May 8	30.0	9.7	41	5.2	306	179	
May 9	29.0	10.2	39	6.4	371	226	Slight cholemia
May 10	30.0	9.7	40	6.5	381	228	Dog normal

values most conspicuous on the fourth day after the injection. It is interesting to note the initial stimulus on the first 2 days after giving this large dose. The action of phosphorus is very different when contrasted with chloroform. We may say that the type of cell injury is just as different as is the fibrin reaction recorded here. The histological changes parallel in a striking fashion the reaction on the part of the blood fibrin. Cell necrosis is promptly established and very conspicuous in chloroform poisoning. Fatty degeneration is conspicuous and cell necrosis infrequent; further, the injury does not appear promptly after administration of phosphorus. All of this fits in perfectly with the reaction observed in the blood fibrin values. The interdependence of liver injury, liver function and the blood fibrin levels is indeed striking and must have much significance as regards the source of supply of fibrinogen in the body.

TABLE 46

Hydrazine sulfate poisoning—toxic dose

Dog 19–124. Mongrel, male. 37 lbs.

DATE	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
			Average	100 cc. Plasma	100 cc. Blood	
	cc.	*per cent*	*mgm.*	*mgm.*	*mgm.*	
April 29	9.8	45	6.0	368	203	Mixed diet
April 29	Hydrazine sulfate—80 mgm. per K.—subcutaneously					
April 30	9.5	58	6.4	417	173	Very toxic. Refuses food
May 1	9.8	63	4.2	271	103	Less toxic. Cholemia
May 2	10.0	60	3.6	225	90	Cholemia marked. Clinically improved
May 3	10.0	58	2.7	167	70	Cholemia marked
May 6	9.2	50	4.4	257	128	Dog normal. Cholemia marked
June 8	9.0	45	4.6	274	150	Cholemia—trace
June 12	10.0	39	5.2	302	184	Weight 33 lbs.
June 18	9.8	47	6.8	421	224	Normal
June 21	9.9	46	8.8	521	279	Weight 37 lbs.

Hydrazine sulphate (table 46) is a well-recognized liver poison which in its effects upon the liver resembles phosphorus. We note that the fibrin reaction to a large toxic dose is very similar to that given for phosphorus (table 45). There is the slow steady fall to the lowest level on the fourth day after administration of the drug. What appears to be a stimulus to fibrin production on the first day is shown by the hematocrit to be the result of blood concentration. The dog was clinically very sick, refused food, vomited and drank much water. In spite of this we note a remarkable rise in the red cell hematocrit which may indicate plasma concentration in spite of liberal fluid intake. This point merits further study. Cholemia is marked during the severe intoxication and present even after clinical symptoms have cleared up.

TABLE 47

Turpentine abscess—chloroform anesthesia following day—fasting period

Dog 19–1. Dog, old, male bull

DATE	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
			Average	100 cc. Plasma	100 cc. Blood	
	cc.	*per cent*	*mgm.*	*mgm.*	*mgm.*	
January 27	9.0	46	8.1	487	265	Weight 35.3 lbs.
January 27	Turpentine—1 cc. subcutaneously					
January 28	9.7	60	10.3	647	261	
January 28	Chloroform anesthesia—75 minutes—3rd day of fasting					
January 29	9.1	53	12.8	788	373	Fasting
January 30	9.0	53	15.9	981	468	Dog toxic. Cholemia Weight 32.6 lbs.
January 31	9.2	52	15.2	918	441	Large sized abscess
February 1	9.6	54	15.6	975	440	Fasting
February 2	9.4	55	13.6	841	376	Abscess full
February 3	9.0	56	11.6	726	323	Abscess ruptured
February 4	9.1	58	11.8	745	311	Good drainage Dog brighter. Weight 29.2 lbs.
February 5	9.3	56	12.0	746	328	Crackermeal and rice diet, daily
February 6	9.6	51	10.1	610	299	Dog active
February 7	10.0	49	8.7	520	265	Weight 30.0 lbs.
February 8	8.8	47	9.3	561	300	Abscess healed Weight 28.0 lbs.
February 9	9.2	47	9.0	551	258	Dog normal
February 10	8.0	46	7.3	438	236	Weight 30.0 lbs.

Abscess plus chloroform injury—combined reaction. Tables 47, 48 and 49 give experiments in which we can study the combined reaction following a depressing chloroform injury and the stimulus of a sterile abscess. In the first experiment (table 47) the turpentine is given on the second fasting day and the 75 minute chloroform injury on the following day. We are glad to refer to a control experiment on this same dog in table 42. The same chloroform injury was followed by a definite and prolonged fall in fibrin values. It was noted however that this old dog was more resistant to chloroform injury than the average adult dog and we believe this is a constant reaction under uniform experimental conditions (2). There is cholemia which assures us of a considerable liver injury. In spite of this injury we note the usual high rise in blood fibrin values. The rise is well sustained but perhaps not at quite the high level to be expected in an uncomplicated abscess. There is quite a fall in fibrin values before the abscess rupture and this is not often observed in controls without liver injury. From this experiment one might conclude that even the injured liver can respond normally to a stimulus for fibrinogen production (abscess) but we believe this is not literally true. We believe the evidence is that the injury was not sufficiently great to paralyze the liver reaction. It may also be considered that some reserve supply of fibrinogen is drawn upon in this emergency. It seems possible that a great stimulus (abscess) may call out a fibrinogen production which under less stress would not be furnished by the liver.

The second experiment (table 48) shows a simultaneous chloroform injury and abscess stimulus. In this reaction the depressant chloroform injury dominates the situation and we see the usual low level on the second day. In an uncomplicated chloroform poisoning we would expect these low values to persist for several days (table 43), but we see a sharp rise to levels 50 per cent above normal. This would indicate that a great stimulus is able to lift the blood fibrin level considerably above normal even in the face of a considerable liver injury, which is undergoing the usual repair. The liver repair during fasting is known to be considerably delayed and would not be complete for ten days or longer (3). We do not see how this reaction can be explained on the basis of a reserve of fibrinogen in the body because any such reserve would be thrown in *early* in the reaction when the fibrin levels are falling or at the lowest point on the first and second days. This is strong evidence we believe that the injured (or repairing) liver may be associated with low fibrin values but, given a very strong stimulus (abscess) and a non-lethal injury, one may observe a strong reaction in fibrinogen production on the part of the injured liver. In other words, a great stimulus is still able to spur the liver which has suffered a non-lethal injury to great efforts in fibrinogen production. Further, the evidence is that a *low fibrin value* is not as great a stimulus to fibrinogen production as is the presence of *injured tissue* outside of the liver in the body.

TABLE 18

Turpentine abscess—chloroform anesthesia same day—fasting period
Dog 19-2. Dog, young, female brindle bull

DATE	WEIGHT	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
				Average	100 cc. Plasma	100 cc. Blood	
	lbs.	*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	
January 28		9.8	46	6.8	404	218	
January 28	Chloroform anesthesia—75 minutes. Turpentine—1 cc. subcutaneously. 3rd day fasting						
January 29	24.0	9.0	57	3.4	210	91	Dog quiet. Cholemia marked
January 30	23.4	8.9	61	2.4	154	61	Cholemia +. Abscess fluctuant and small
January 31		9.1	66	3.6	238	81	Cholemia marked Abscess ruptured
February 1	21.7	10.5	58	5.7	346	145	Dog not toxic
February 2		9.8	51	5.7	344	169	Abscess draining well
February 3	20.8	10.0	49	7.8	466	238	Fasting
February 4		9.6	48	8.6	513	267	
February 5	20.2	10.0	49	10.0	595	303	Abscess not draining Fed crackermeal and rice, daily
February 6		9.4	45	10.7	579	317	Cholemia
February 7	20.2	10.0	44	9.4	561	307	Cholemia Drainage poor
February 8	19.7	9.0	41	9.7	576	340	Abscess healing
February 9		10.0	40	8.7	514	303	Diarrhea Abscess healed
February 10	20.4	6.4	42	6.8	428	248	Dog active and well
February 11	20.6	9.2	47	7.2	433	231	Dog active and well
February 12	20.7	9.1	44	6.1	361	202	Dog active and well

We wish to note also the uniform increase in the red cell hematocrit during the acute period of intoxication associated with abscess formation. This same rise in red cell hematocrit is noted in many other similar intoxications and is usually accepted as an indication of loss of circulating fluids. It may be a positive reaction indicative of cell injury and affinity of such injured protoplasm for fluids. We believe it is a complicated reaction of much importance and hope to report further studies of these phenomena.

In the third experiment of this series, the chloroform was given on the day before the abscess (table 49). Again we note low values on the second day after the chloroform, due certainly to extensive liver injury but there is a sharp rise in fibrin values immediately after the initial depression. This experiment confirms that in table 48 and supports the conclusions drawn from that experiment. There is opportunity for speculation when one reflects that the liver injured by chloro-

TABLE 49

Chloroform anesthesia—turpentine abscess following day—fasting period
Dog 19–65. Spitz, male

DATE	WEIGHT	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
				Average	100 cc. Plasma	100 cc. Blood	
	lbs.	*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	
February 8	22.6	9.8	61	5.4	341	132	
February 8	Chloroform anesthesia—75 minutes—3rd day of fasting						
February 9	21.3	10.1	59	3.5	216	89	Fasting
February 9	Turpentine—1 cc. subcutaneously						
February 10	20.6	10.3	59	2.6	160	65	Dog dull. Abscess forming
February 11	20.1	10.0	59	5.8	360	148	Dog toxic. Cholemia
February 12	20.0	8.8	56	6.5	408	180	Dog toxic. Cholemia marked. Abscess 10 × 10 × 3 cm.
February 13	19.0	9.9	54	6.2	378	174	Condition same
February 14		10.3	53	7.3	441	206	Abscess ruptured
February 15	19.1	10.0	55	9.1	555	250	Abscess draining well. Dog bright. Cholemia
February 16	18.5	10.0	55	7.0	427	192	Fasting
February 17	18.2	10.0	53	7.0	424	199	Fed crackermeal and rice, daily Drainage poor
February 18	18.3	10.0	50	5.5	330	165	
February 19	18.6	7.6	47	4.8	297	156	Abscess smaller
February 21	18.2	8.2	43	5.1	306	176	Cholemia slight
February 22	20.0	8.8	43	5.0	300	171	Dog normal

form may be associated with low fibrinogen production but not by reason of necessity, as it is clear that a great stimulus can overcome this lethargy. Does the liver carry this low output during the period of repair and regeneration of new liver cells in order to spare its cell activity or because the same material may contribute to both processes?

TABLE 50

Eck fistula—chloroform anesthesia—stimulating dose
Dog 19–6. Bull terrier, male

DATE	WEIGHT	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
				Average	100 cc. Plasma	100 cc. Blood	
	lbs.	*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	
February 25	30.0	9.6	59	5.5	342	139	Fasting
February 26	29.0	9.0	54	6.0	373	170	Fasting
February 27	28.5	8.7	60	6.0	388	155	Fasting
February 28	28.0	8.4	61	4.8	314	122	Mixed diet until March 4
March 4	29.0	8.1	60	6.6	433	171	
March 4	Operation—Eck fistula						
March 6		10.0	66	10.1	653	222	Milk and bread diet
March 10	27.4	9.6	58	6.1	381	159	Dog normal
March 11	27.4	10.2	56	4.5	275	122	
March 17	28.1	10.0	54	5.2	316	145	
March 20	26.5	10.1	52	5.3	320	154	
March 21	27.0	10.4	54	4.6	268	128	Dog very active
March 24	25.2	10.1	56	4.2	257	113	Mixed diet
March 25	Chloroform anesthesia—45 minutes						
March 26	26.0		58	5.2	286	120	Dog active
March 27	26.0		59	5.8	365	151	Cholemia
March 28	26.6		55	5.2	318	143	Cholemia—trace
March 29	27.0		58	5.2	320	135	Diarrhea
March 31	26.2	9.8	58	4.9	310	128	Dog active

Tables 50 and 51 give a series of fibrin determinations on a normal dog before and after a successful Eck fistula operation. The autopsy record shows that the operation was successful and the fistula between the portal vein and vena cava was of large size. The portal vein at the hilum of the liver above the fistula was completely occluded by ligature. All portal blood, therefore, was shunted around the liver direct into the right auricle. It is well known that under these conditions the liver undergoes distinct atrophy and usually if not always presents a central fatty degeneration. There is obvious impairment of function but

TABLE 51

Eck fistula—abscess and chloroform reactions
Dog 19–6. Bull terrier, male

DATE	WEIGHT	TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN Average	FIBRIN 100 cc. Plasma	FIBRIN 100 cc. Blood	REMARKS
	lbs.	*cc.*	*per cent*	*mgm.*	*mgm.*	*mgm.*	
April 1	26.0	10.1	50	5.8	346	175	Bread, milk and bones
April 2		10.1	48	5.4	320	168	
April 3	26.7	10.1	45	6.1	360	198	Normal
April 4		10.0	46	6.1	361	195	
April 4	Turpentine 0.5 cc. subcutaneously						
April 5	25.9	10.2	51	7.7	463	223	Abscess 11 × 8 × 3 cm.
April 6		10.5	50	10.5	625	313	Abscess fluctuant
April 7	24.8	10.2	46	9.7	573	309	
April 8		10.1	46	8.5	495	299	Abscess opened
April 9	25.0	9.6	40	7.2	417	270	
April 10	25.7	9.3	41	7.3	430	256	Abscess draining
April 11	26.2	10.4	44	7.6	450	278	
April 12	27.0	9.4	45	6.4	381	211	
April 14	27.1	9.6	41	6.4	376	223	Abscess draining
April 15	27.0	9.9	44	5.8	343	191	
April 16	26.0	10.1	42	6.1	356	208	
April 17	26.2	9.8	40	4.4	258	154	Abscess healing slowly
April 18	26.2	9.4	41	5.2	307	180	Drainage good
April 19	26.1	10.0	43	5.5	323	184	
April 23	25.0	10.0	47	5.1	303	161	Abscess healed Dog active
April 29		10.0	49	6.0	359	183	
April 29	Chloroform anesthesia—75 minutes						
April 30	27.0	9.7	53	5.8	353	168	Dog normal
May 1	24.0	9.7	52	2.2	133	61	Cholemia marked Dog very toxic
May 2	23.0	9.7	58	2.4	151	64	
May 3	23.0	9.6	61	2.6	165	64	Dog comatose Sacrificed

the condition is not a simple one and the cause of the intoxication which develops sooner or later is not at all understood (13).

The fasting level of this normal dog is constant and there is a rise on the mixed diet (table 50). Following the operation we observe on the second day a marked rise in fibrin values. This is surely to be explained by the operative trauma and wound reaction. The fibrin level after the operation is much below that before operation and this may have significance, perhaps indicating functional impairment. Not sufficient data are at hand to settle this point.

A stimulating dose of chloroform anesthesia gives the expected reaction (table 50). Compare this reaction with that in table 41 where the same type of chloroform injury shows a more striking stimulus and rise in fibrin values. It is to be noted that the dog in table 41 was fasting and in table 50 was not. The extent of the liver injury cannot be estimated and may have been much less than in table 41. The presence of the Eck fistula may further modify the reaction.

Dog 19–6—Eck fistula—autopsy. Autopsy was performed immediately after death and all organs were examined. Only the liver findings will be recorded. The Eck fistula was perfect and the ligature above it at the hilum of the liver completely occluded the portal vein. The liver was smaller than normal and tough. The centers of the liver lobules were yellowish. Microscopical sections show the usual central atrophy in the liver lobules. There are small central hyalin necroses and many wandering cells. Fatty degeneration is marked and involves 30 to 40 per cent of the lobule. The liver cells are "foamy" and severely injured. This necrosis and fatty degeneration involve about one-third of the remaining liver cells. This degeneration is not sufficient in itself to cause death. The remaining liver cells appear normal. There are scattered mitotic figures. Some liver cells show hyalin bile-stained plugs in the bile canaliculi.

Table 51 continues the observations on the same Eck fistula dog of table 50. A sterile subcutaneous abscess is produced and gives about the expected reaction. There is very little difference between the stimulus reaction in the Eck fistula when compared with a normal dog of similar type and weight (table 52). The development of the abscess in each dog calls out the characteristic reaction and prompt rise in fibrin values. There is a prompt fall when the abscess is incised.

Finally the dog (table 51) is given a chloroform anesthesia of 75 minutes—a depressing dose for a normal fasting dog. The Eck fistula dog was not fasting but the injury done the liver was considerable as shown by the autopsy record. The second day after the chloroform we observe a considerable fall in the fibrin values maintained until fatal intoxication supervenes. The clinical picture did not suggest a fatal late chloroform poisoning and the autopsy confirms this. It is probable that the usual Eck fistula intoxication was responsible for the fatal intoxication, perhaps precipitated by the liver injury due to the chloroform.

A note should be made concerning the healing of these aseptic abscesses. In the normal dog such abscesses if properly drained will heal in six to nine days. This abscess (table 51) in the Eck fistula dog did not heal with the best of drainage until the 16th day. The abscesses in dogs poisoned by chloroform also were delayed in healing and required 13 to 14 days. We have observed that laparotomy wounds in Eck fistula dogs may heal very slowly with sluggish granulation tissue. The same observation concerning laparotomy wounds has been made by Davis and Whipple (4) in their series of dogs poisoned by chloroform. Clark (1) has observed that wounds heal more rapidly when an animal is on a rich protein diet. It is at least possible that other substances besides fibrin which take part in the ordinary wound healing may be influenced directly by the liver injury and food factors.

TABLE 52

Control for Eck fistula—sterile abscess

Dog 19–66

DATE	WEIGHT	R. B. C. HEMATOCRIT	FIBRIN			REMARKS
			Average	100 cc. Plasma	100 cc. Blood	
	lbs.	*per cent*	*mgm.*	*mgm.*	*mgm.*	
April 29	27.0	57	4.5	281	121	Mixed diet
April 29	Turpentine—0.5 cc. subcutaneously					
April 30	27.0	60	5.6	413	167	Abscess 8 × 10 × 3 cm.
May 1		57	11.2	697	298	Dog toxic
May 2	28.8	49	11.0	662	335	Abscess tense
May 5	29.0	51	8.1	478	239	Abscess opened
May 6		44	7.6	451	252	Good drainage. Dog active
May 7		47	7.4	443	233	
May 8	28.6	50	6.0	361	181	Abscess healing
May 9	28.3	44	5.5	336	187	
May 10	28.3	48	5.5	330	170	
May 12	27.0	47	5.0	298	157	Abscess healed
May 14	27.4	50	5.6	336	157	
May 16	27.4	44	5.7	337	187	
May 18		51	6.8	425	209	
May 21	28.4	48	4.6	275	143	

The last table (no. 53) presents some data on human patients. These figures have some interest when compared with the figures for the normal and abnormal dogs. This series of human cases will be extended in the near future. The normal patients though few in number are very

TABLE 53

Human cases—normal—influenza—blood and liver diseases

TOTAL VOLUME BLOOD	R. B. C. HEMATOCRIT	FIBRIN			DIAGNOSIS	REMARKS
		Average	100 cc. Plasma	100 cc. Blood		
cc.	*per cent*	*mgm.*	*mgm.*	*mgm.*		
8.8	47	5.4	328	175	Normal	
9.6	51	6.0	364	178	Normal	
9.4	51	5.5	333	163	Normal	
10.7	45	5.4	316	175	Normal	
Average..........			335	172		
6.5	85	17.1	1710	263	Influenzal pneumonia	Fatal. Blood urea 96
10.2	43	16.9	987	562	Influenzal pneumonia	Fatal. Blood urea 53
11.0	50	18.6	1099	549	Influenzal pneumonia	Fatal. Blood urea 65
9.8	33	12.6	725	489	Influenzal pneumonia	Not fatal. Blood urea 35
9.9	51	22.6	1366	676	Influenzal pneumonia	Blood urea 51
11.1	59	10.2	689	282	Influenzal pneumonia	Very toxic. Blood urea 53
9.5	55	12.2	753	337	Influenzal pneumonia	Toxic. Blood urea 49
9.4	52	11.8	718	344	Influenzal pneumonia	Very toxic
8.9	34	25.4	1482	992	Influenzal pneumonia	Fatal
11.3	70	4.6	294	88	Polycythemia vera	Cholemia, slight
5.9	42	10.5	680	394	Jaundice	Cholemia +++
9.0	33	11.6	677	461	Suspected cirrhosis	
9.5	33	7.5	431	290	Cirrhosis	Autopsy showed cirrhosis
8.5	58	8.6	546	222	Purpura	Petechial hemorrhages
13.1	18	8.4	458	378	Acute lymphatic leukemia	

uniform in fibrin values. The influenza cases present some remarkably high values with and without blood concentration. The abnormal blood and liver cases are too few in number to permit any generalization. It is suggestive that this case of polycythemia shows a low fibrin level and a somewhat similar finding was recorded in experimental plethora or polycythemia (table 29).

Discussion of sources of fibrinogen supply. A considerable number of factors which stimulate fibrin production or at least cause a rise in fibrin values has been mentioned. These include food factors, hemorrhage, any type of inflammation or tissue injury and even certain general intoxications without obvious cell injury. We may dismiss with a word the theory that fibrin is formed in the bone marrow as it has been shown that essential diseased conditions of the red marrow are not associated with blood fibrinogen abnormalities. Even fatal aplastic anemia shows no fall in fibrin values (8).

It has been thought that the intestinal tract was the essential source of fibrinogen but the extirpation of the entire small intestine does not disturb blood fibrin values and the extensive injury of the epithelium of the small intestine (x-ray experiments, table 39) calls out a very considerable fibrinogen over-production just like tissue injury elsewhere in the body.

Simple hemorrhage causes a distinct over-production of fibrinogen and this is not to be explained by a dilution of the fibrin values alone. We are able to dilute fibrin values in the circulation without causing any reaction if we use acacia solutions or fresh serum. Probably the changes in blood volume are important factors and a *shrinkage* of plasma volume is very apt to bring out an over-supply of fibrinogen. We have good evidence (table 29, paper III and table 53, polycythemia) that *plethora* inhibits the fibrinogen production or at least is associated with abnormally low levels.

Evidence has been noted which indicates a constant daily consumption of fibrin in the normal body (paper III). Also we are familiar with the fibrin deposit which is conspicuous and probably continuous in acute inflammation (pneumonia, peritonitis, abscess and so forth). It is very probable that actual tissue necrosis (hyalin liver or cell necrosis, infarcts and so forth) is associated with considerable deposit of fibrin. Therefore, if the supply of fibrin is cut out and tissue injury is present, we may observe a very sharp drop in fibrin values (acute and severe chloroform liver necrosis).

It is more than possible that there may be certain reserve supplies of fibrinogen in the body and the intestinal tract may be important in this respect (8), (5) but it is unlikely that these reserves can supply any very great amount of fibrinogen over long periods of time.

The only condition which without exception causes or is associated with low fibrin values is *acute extensive liver injury* (chloroform, phosphorus and so forth). The uniformity of this reaction is of great significance and especially the remarkable parallelism which is demonstrable between the extent of the liver injury and the depression in fibrin values. Slight liver injury will cause a rise in blood fibrin values like tissue injury elsewhere in the body but when the injury is extreme and acute we invariably see a prompt fall which usually reaches its lowest level on the second day following the chloroform. This is the period of maximum liver injury and edema with minimum liver repair.

A combination of a toxic or depressant dose of chloroform with a stimulus to fibrinogen production (abscess) gives some very suggestive data. The initial fall (48 hours) in fibrin values due to extensive liver necrosis cannot be obliterated by the abscess stimulus, but the period following this shows the usual rise in fibrin values, persisting until the abscess is drained. This is very different from the usual low level following chloroform poisoning which persists throughout the week or more of liver repair. Evidently then, the repairing or regenerating liver is usually associated with low fibrin values—this may be to conserve cell energy or construction material—but may be spurred to great over-production of fibrinogen by an appropriate stimulus (abscess). This observation makes it very difficult to postulate active fibrinogen production or even extensive reserve supply outside of the liver. It would seem that this emergency reserve would be most obvious at the first of the period when the fibrin values are lowest and if present this reserve is quickly exhausted. We conclude, therefore, that all the evidence points to the liver as the only actively productive source of fibrinogen although there may be certain limited reserve supplies in other body tissues.

SUMMARY

Tissue injury and inflammation exert a powerful stimulus upon fibrinogen production and cause prompt and notable increase in fibrin values. Bacteria are not directly concerned in this reaction, which is identical whether a sterile or septic inflammation is present.

Toxic proteoses cause a fibrinogen reaction which presumably is due to tissue injury although we find no histological evidence of inflammation or tissue injury.

Injury by the Roentgen rays whether over the abdomen (lethal) or over the thorax (non-lethal) gives a prompt rise in blood fibrin values. This thorax exposure with injury of red marrow and lymph cells shows the influence of *cell injury* quite apart from any true inflammation.

Acute infections in dogs (table 40) and humans (table 53) give the familiar stimulus to fibrinogen production and show probably the highest values in blood fibrin.

Small doses of liver poisons (chloroform, phosphorus and hydrazine) are stimulating and cause a *rise* in fibrin values just as tissue injury elsewhere in the body.

Large doses of liver poisons are depressing and if sufficient liver parenchyma is injured invariably show a fall in fibrin values.

A combination of liver injury (depression) with an abscess reaction (stimulus) gives information of value. If liver injury is severe, there will be the initial fall in fibrin values. After the second day of injury, the liver which is regenerating can be stimulated to great production of fibrinogen by appropriate stimuli (abscess). Without this stimulus the liver will maintain only a low fibrin level during its period of repair. The significance of this observation is discussed.

The Eck fistula liver in general reacts like the normal liver in its fibrinogen production. There are minor differences from normal and the Eck fistula intoxication probably has no influence upon fibrin values.

Sterile abscesses heal more slowly in Eck fistula dogs or those poisoned with chloroform than in normal controls. It is possible that fibrinogen or other substances fabricated in the liver play a rôle in normal wound healing.

Plethora in humans (table 53—polycythemia) and in dogs (paper III) experimentally produced is often associated with low fibrin values. This may be a simple reciprocal reaction contrasting with the familiar fibrinogen regeneration resulting from hemorrhage or falling blood volume. Mere dilution of fibrinogen values does not necessarily stimulate fibrinogen production (serum or acacia injections).

Tissue injury is the most powerful single stimulus to over-production of fibrinogen and far exceeds the stimulus of a low blood fibrin level.

There may be certain reserves of fibrinogen or its parent substance held in certain body tissues other than the liver but this reserve is probably not large. We conclude that all available data point to the liver as the only potential source of fibrinogen in the body.

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[Reprinted from THE JOURNAL OF EXPERIMENTAL MEDICINE, February 1, 1922,
Vol. xxxv, No. 2, pp. 187–202.]

ROENTGEN RAY INTOXICATION.

I. UNIT DOSE OVER THORAX NEGATIVE—OVER ABDOMEN LETHAL. EPITHELIUM OF SMALL INTESTINE SENSITIVE TO X-RAYS.

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(Received for publication, August 16, 1921.)

The papers of this series form a part of the general program of study of the "non-specific intoxications" which is being carried forward in this laboratory. They are concerned with an investigation of the systemic intoxication which develops after a suitable exposure to large doses of hard Roentgen rays. These papers follow along the logical development of this study, based on the beginnings made by Hall and Whipple (3). Reference to the paper of Hall and Whipple is necessary for a review of some of the rather extensive literature of this subject and for a discussion of many of the fundamental problems related to Roentgen ray intoxication.

This Roentgen ray intoxication, we believe, is a true "non-specific intoxication" and deserves thorough study in part for this very reason. Too little study has been directed toward an understanding of the "non-specific intoxication" which often is a very important factor in many of the so called specific infections. This Roentgen ray intoxication closely resembles the intoxication of intestinal obstruction, another important "non-specific intoxication." We shall point out some important differences between the intoxication due to the Roentgen rays as contrasted with intestinal obstruction, but there remain many striking similarities as emphasized by Hall and Whipple (3).

We believe our experiments make it clear that the fundamental thing in the systemic intoxication due to the Roentgen rays is a *primary injury of the epithelium of the small intestine.* This was suspected by Hall and Whipple but not conclusively proven. Our experiments in normal dogs under controlled conditions appear convincing when exposures given over the thorax are contrasted with the same exposures over the abdomen. For example, a unit dose (350 milliampere minutes) given over the thorax (abdomen shielded) causes no clinical disturbance in the dog. A subsequent exposure to the same unit dose in the same dog given over the abdomen (thorax shielded) will cause fatal intoxication and death in 4 days. For a normal 30 to 40 pound dog, we may say that 350 milliampere minutes given over the abdomen is a minimum lethal dose.

The outstanding abnormalities in the abdominal exposures are the remarkable necroses observed in the small intestine. Large areas may show complete necrosis and disappearance of the intestinal epithelium covering the villi and lining the crypts. The villi are left as naked polyps made up of stroma, vessels, and a few wandering cells. The inflammatory reaction is not as intense as would be expected under such conditions with complete removal of the covering epithelium. This important point will be discussed in detail in a subsequent paper.

Regaud, Nogier, and Lacassagne (5) described the chronic gastrointestinal lesions in dogs and noted especially gastric atrophy and intestinal perforation. Fromme (2) noted especially the clinical symptoms of gastrointestinal disturbance in guinea pigs and mice. Denis, Martin, and Aldrich (1) noted in rabbits the toxic effect of abdominal radiation and certain abnormalities in the intestines which they state are secondary to an unknown toxic factor.

Method.

The procedure is identical in all experiments, unless otherwise indicated. Normal dogs are used throughout and are kept in standard metabolism cages which have sharply pitched floors to facilitate the collection of urine. To insure the acidity of the cage collections, about 1 cc. of glacial acetic acid is added to the cage collecting bottle at the beginning of each 24 hour collection. The dogs are catheterized daily at 10 o'clock. The cage collections, cage washings, bladder washings, and catheterized urine are mixed and diluted to a unit volume—usually 2 liters. The total nitrogen is then done in duplicate by the Kjeldahl method. About 300 cc. of warm water is given daily by stomach tube as a routine procedure after the dog is catheterized. Water is available at all times in the cage.

Dogs are given a subcutaneous injection of morphine shortly before exposure to radiation. A medium hard Coolidge tube is used with the target (anode or anticathode) set at a constant distance of 10 inches from the skin. At this distance the effective radiation for this machine covers a circle approximately 6½ inches in diameter. The current strength used is either 7.5 or 8.0 milliamperes. The E.M.F. varies considerably in different experiments from 85 kilovolts to 103 kilovolts. The spark-gap backing up these voltages varies also, measuring from 8½ to 10 inches between sharp points. The amount of radiation is calculated as the product of the current strength in milliamperes and the time of exposure in minutes and is expressed as milliampere minutes. In all these experiments the standard autotransformer x-ray equipment of the University of California Hospital is used. Aluminum filters (2 mm.) are used to cut out the softer rays. The various parts of the dog were effectively protected from radiation, when it was so desired, by sheets of lead-foil approximately 2 mm. thick, or by lead "rubber," approximately 5 mm. in thickness.

The body of the dog is marked off roughly into two parts by a transverse line at the level of the xiphisternum. The part between this line and the base of the neck is designated as the thorax, that below as the abdomen. Each of these parts is then divided into an upper and lower half. Each upper and lower segment of the abdomen or thorax is radiated from the lateral and midventral aspects. Thus the ventral portion of the abdomen or thorax is exposed to radiation over six different areas whose contiguous edges overlap somewhat. Usually one exposure is given over the middorsal region of the thorax or abdomen, causing the total dosage to be divided up into seven parts, which, however, are spread rather diffusely over the part of the body involved. All dogs are sacrificed under chloroform or ether anesthesia by bleeding.

EXPERIMENTAL OBSERVATIONS.

Some of our typical experiments are tabulated below and make several points quite clear. However, we wish to refer to other experiments of similar nature given in subsequent papers of this series. These experiments are all in agreement and indicate that enormous doses of Roentgen rays may be given over the thorax without causing any clinical reaction in a normal dog. Dogs have been given amounts of radiation varying from 300 to 512 milliampere minutes over the thorax without any clinical symptoms. One dog (No. 19–117) was given 350 milliampere minutes over the thorax on three separate occasions with intervening periods of 2 to 3 weeks; all this without any apparent cumulative effects or clinical reaction. There is usually a slight transient leucopenia and there may be a very slight rise in total nitrogen excretion. After 2 or 3 weeks, the skin shows loss of hair and pigmentation as a result of the exposures. The ribs of the animal examined at autopsy show a considerable loss of marrow cell elements. We have been able to recognize no other results from these large doses of x-rays over the thorax.

Dog 19–78 (Table I) is a typical example of a complete experiment to show that a unit dose of x-rays is clinically inert over the thorax but lethal when given over the abdomen. The thorax dose causes a definite leucopenia but no clinical disturbance. That the second dose does not cause as much of a leucopenia, we believe, may be due to the tissue injury in the small intestine.

The radiation over the abdomen causes a typical clinical reaction and the review of this case will suffice for all subsequent experiments. This clinical reaction is remarkably constant and is described in detail below (Dog 19–78). The latent period of 24 to 36 hours is noted as usual and will be discussed later. The rise in total urinary nitrogen is not great but is significant. The figure for the last day includes a certain amount of fecal contamination.

Dog 19–78. Clinical History and Autopsy (See Table I).

Jan. 27. The dog is isolated on fasting diet. The animal is in good condition and quite active.

Jan. 29. The abdomen is covered with lead-foil well up to the level of the diaphragm. The thorax is exposed in seven areas, two midventral, two on each side, and one over the midscapular region. Each area exposed covered a patch of skin approximately 5 inches square. Morphia ($\frac{5}{8}$ gr.) is given to keep the dog quiet during the exposure. The current strength is 7.5 milliamperes at an E. M. F. of 100 kilovolts with the spark-gap set at 9 $\frac{9}{16}$ inches. 2 mm. of aluminum filter is used and the tube is set at a target-skin distance of 10 inches.

TABLE I.

Roentgen Radiation.

Unit Dose Given over Thorax and Subsequently over Abdomen.

Dog 19–78. Adult, female.

Date.	Weight.	White blood corpuscles per c. mm.	Urine.		Diet.	Remarks.
			Volume.	Total N.		
	lbs.		*cc.*	*gm.*		
Jan. 28	26.0	—	—	—	Water.	Good condition.
" 29	26.0	7,600	—	—	"	
Jan. 29	X-rays, 350 milliampere minutes, given over thorax. Abdomen shielded.					
Jan. 30	25.8	8,000	—	—	Water.	Dog normal.
" 31	24.5	4,100	—	—	"	
Feb. 1	23.8	4,600	—	—	"	Dog normal.
" 2	23.3	4,600	—	—	"	
" 3	23.0	—	—	—	Mixed.	Dog normal.
" 8	27.0	5,400	—	—	"	
" 21	28.3	7,400	—	—	"	Dog normal.
	Experiment ended.					
Mar. 15	28.9	7,800	—	—	Water.	Dog normal.
" 17	27.8	7,600	260	2.74	"	
" 18	27.3	8,000	305	2.68	"	Dog normal.
Mar. 18	X-rays, 350 milliampere minutes, given over abdomen. Thorax shielded.					
Mar. 19	26.8	6,400	290	2.83	Water.	Dog normal.
" 20	26.6	7,000	265	2.74	"	Inactive.
" 21	25.9	7,200	240	3.00	"	Vomitus and diarrhea.
" 22	25.0	3,900	—	5.63	"	Moribund.
	Chloroform anesthesia and autopsy at once.					

Jan. 30. Dog remains normal during entire experiment (see Table I). There was a slight transient leucopenia. Dog was on mixed diet until the second experiment, also given in Table I.

Mar. 15. The dog is active and in good condition.

Mar. 18. Dog given ½ gr. of morphia 1 hour before exposure to radiation. The thorax and head and neck are thoroughly screened with lead-foil down to the tip of the xiphisternum. Seven areas are exposed, two midventral, two on each lateral surface, and one midlumbar. The current strength is 7.5 milliamperes at an E. M. F. of 95 kilovolts, backed by a 9½ inch spark-gap. The radiation is filtered by 2 mm. of aluminum. The skin-target distance is 10 inches. There are no immediate after effects from exposure to 350 milliampere minutes.

Mar. 19. The dog is bright and active.

Mar. 20. Slight evidences of intoxication are manifested by the dog—one soft, yellow, watery stool.

Mar. 21. The intoxication becomes marked in the afternoon. The diarrhea has progressed from brownish, watery material to tarry, black mucous or bloody mucous material with a very foul odor. Vomitus increases in amount and frequency during the day.

Mar. 22. The animal is very weak and dull. In the afternoon, the dog is completely prostrated. The stools have become very frequent and of large volume. The pulse is irregular and thready. The temperature has risen to 40.2°C. The respirations are rapid and irregular. The animal is practically moribund and is sacrificed under chloroform anesthesia.

Autopsy.—Performed immediately after death by chloroform. The hair over the thorax is thinned. It is absent over the sternum where the skin is somewhat pigmented from previous exposures to radiation. Pleural cavity, heart, and lungs are normal. Peritoneal surfaces are normal. Spleen is somewhat granular in appearance, hard and firm, normal in size. On section, the trabeculæ stand out. Mesenteric glands slightly enlarged and moist—somewhat pinkish in color. Kidneys normal in size and appearance. Bladder contracted; it shows a few ecchymoses in its mucosa (catheter). Liver is normal. Adrenals are somewhat enlarged; apparently normal except for a small necrotic mass in the left adrenal.

Stomach contains gas and about 20 cc. of a bile-stained fluid. The mucosa is pale and normal.

Duodenum is somewhat thick-walled and spastic. The lumen contains many large, round worms and a small amount of reddish-black mucus. Beginning sharply at a point approximately an inch distal to the *pyloric valve*, the mucosa of the *duodenum* presents a dark red glassy appearance. On this background run several longitudinal folds with bright red hemorrhagic crests. Numbers of small bright red petechiæ show up between these plicæ on the dark, glassy surface.

The reaction in the *jejunum* seems less intense, for here the mucosa is paler and the plicæ are not so conspicuous though they can be distinctly made out.

Ileum contains a small amount of reddish-black mucous material similar to the stools, and a few round worms. The intestinal wall is thin and rather flaccid and on close inspection the intensely hemorrhagic mucosa surface seems to have been denuded of most of the villi as the velvety appearance is lacking. The longitudinal folds with bright red hemorrhagic crests are conspicuous. Peyer's patches seem to be stripped of their villous covering so that they show up readily as speckled oval patches. The ileocecal valve marks an abrupt end of this extensive injury.

Colon mucosa is pale, though a few longitudinal, hemorrhagic folds are present. The walls are spastic and the lumen contains a small amount of reddish fluid.

Histological Sections.

Lungs, heart, liver, pancreas, kidneys, bladder, uterus, and stomach are negative. The mesenteric lymph nodes are slightly hemorrhagic and contain some pigment. The spleen is moderately atrophic and contains pigment. In the ovary the ova are apparently dead, though nucleoli and chromatin remnants are still present within the nuclear membrane.

Pyloric duodenum: a section shows the transition from normal intact pyloric villi to collapsed and denuded villi with remnants of dead epithelium and some regenerating epithelial cells undergoing mitosis. Some areas have escaped injury.

Jejunum: destruction and disappearance of the epithelium of villi and crypts; a few crypts remain, otherwise the normal structure is gone. The epithelium around these crypts shows mitotic figures and these are apparently regeneration forms. Large pale epithelial cells stick out into the lumen of the crypts. Their cytoplasm is finely meshed and is decidedly eosinophilic. The nuclei are rather large and pale and frequently contain mitotic figures. A large amount of hemorrhage has occurred in the submucosa, and blood vessels are occasionally found plugged with thrombi of fibrin and platelets. On some areas a very thin sheet of epithelial cells can be seen making an attempt to recover the collapsed villi.

The *ileum* shows extreme injury, with the disappearance of the greater part of the crypt and villous epithelium. Strenuous attempts at regeneration are being made by remnants of the epithelium which shows many mitotic figures and is found clumped in little groups or sheets as if these groups were descendants of one or two surviving cells of the original epithelium.

The *colon* shows some mucoid degeneration and slight necrosis of the tips of a few villi, with evidence of repair.

Dog 19–85. Clinical History and Autopsy (See Tables II and IV).

Dog 19–85 received 300 milliampere minutes over the abdomen (Table IV) which was followed by diarrhea and vomitus and prostration. She recovered. 1 month later she was given 350 milliampere minutes over the thorax with no effect (Table II). A month later (2 months after the first exposure) she was given 350 milliampere minutes over the abdomen with fatal result (Table II).

Mar. 2. Dog fasting since Feb. 28.

Mar. 4. Dog given ½ gr. of morphia 1 hour before exposure to radiation. Lower part of body screened up to xiphisternum by lead-foil 2 mm. thick; 350 milliampere minutes of radiation divided up into the usual seven areas. A current strength of 7.5 milliamperes is used at 103 kilovolts and backed up by a 9⅞ inch spark-gap. A 2 mm. aluminum filter is used. Skin-target distance 10 inches. The total time of exposure is 46.6 minutes. There are no apparent after effects from the x-ray exposure.

Mar. 5 to 8. The dog remains clinically normal.

TABLE II.

Roentgen Radiation.

Unit Dose over Thorax Negative but Subsequent Unit Dose over Abdomen Lethal.

Dog 19–85. Adult, female.

Date.	Weight.	White blood corpuscles per c. mm.	Urine.		Diet.	Remarks.
			Volume.	Total N.		
	lbs.		*cc.*	*gm.*		
Mar. 2	24.7	—	—	—	Water.	Good condition.
" 3	24.4	7,800	355	2.04	"	
" 4	24.1	6,800	310	1.71		Dog normal.
Mar. 4	X-rays, 350 milliampere minutes, given over thorax. Abdomen shielded.					
Mar. 5	23.8	6,600	290	1.90	Water.	Dog normal.
" 6	23.4	8,000	325	1.79	"	" "
" 7	23.2	5,400	325	1.96	"	" "
" 8	22.9	6,800	—	—	"	
" 9	22.8	—	—	—	Mixed.	
	Experiment ended.					
Mar. 30	25.0	—	—	—	Water.	Dog normal.
" 31	24.6	—	440	2.07	"	
Apr. 1	24.1	7,400	325	1.90	"	Dog normal.
Apr. 1	X-rays, 350 milliampere minutes, given over abdomen. Thorax shielded.					
Apr. 2	23.4	7,300	300	2.13	Water.	Dog normal.
" 3	23.1	5,800	290	1.90	"	" "
" 4	22.8	7,200	240	2.07	"	Diarrhea and vomitus.
" 5	22.1	5,400	275	3.16	"	Severe intoxication.
	Ether anesthesia and autopsy at once.					

Mar. 30. Dog has remained normal since the exposure over the thorax. There is some depilitation and pigmentation of previously exposed areas. Dog fasting for past 3 days.

Apr. 1. Animal given ⅜ gr. of morphine 1 hour before exposure; 350 milliampere minutes of radiation spread diffusely over the abdomen in six areas, all ventral. Each area averages approximately 25 square inches. The current strength is 7.5 milliamperes at an E. M. F. of 90 kilovolts, backed up by a 9 inch spark-gap. A 2 mm. aluminum filter is used. Skin-target distance 10 inches. There are no clinical after effects which could be attributed to the radiation.

Apr. 2 and 3. The animal is active and lively.

Apr. 4. There is quite a large volume of yellow, semifluid feces and some bile-stained vomitus throughout the morning. Intoxication becomes more marked in the afternoon. The pulse is regular, but of low tension.

Apr. 5. Greenish-brown, semifluid stools and bile-stained vomitus appear regularly throughout the day. In the afternoon, the stools are tarry and vile smelling. In general, the stools appear to contain digested or partly digested red blood cells, mixed with mucus. The vomitus at no time contains any blood, but is made up of a rather opalescent fluid containing thick, stringy mucus, which at times is bile-stained. The animal is markedly intoxicated, especially during the afternoon. Dog is able to walk but quite weak and ceases to respond voluntarily. The animal is not moribund and might survive, but in all probability would die during the night. Ether anesthesia and autopsy at once.

Autopsy.—Body fat well preserved. Pleural cavity and thoracic viscera are negative. Peritoneal cavity contains no fluid and the peritoneal surfaces are normal. Liver, spleen, pancreas, adrenals, kidneys, and genitourinary tract are negative.

Stomach contains about 20 cc. of bile-stained, frothy mucus, corresponding to the vomitus of the past 2 days. The mucosa is pale and apparently negative.

Small intestine is somewhat spastic and contains a small amount of greenish-brown mucous material.

Longitudinal folds with hemorrhagic crests are conspicuous in the duodenum. These fade out and disappear in the upper jejunum. In both the duodenum and ileum, there are patches or clusters and streaks of tiny ecchymotic specks in the mucosa. The superficial necrosis is not very extensive but stands out in contrast to the normal patches of mucosa in the jejunum and upper ileum, which are pale and velvety and apparently undisturbed.

Colon is negative.

Histological Sections.

Lungs, liver, pancreas, kidneys, adrenals, bladder, and uterus are negative. Ovary: the ova are dead, the nuclear material is broken up, and the cytoplasm is foamy. Spleen: atrophy of the Malpighian bodies and pulp elements; a few fragmented nuclei are seen. Stomach: normal. Duodenum: irregular and patchy injury of villus structures is apparent. In injured areas, there are evidences of mitosis and rapid growth of epithelial cells and some infiltration of the area by polymorphonuclear leucocytes. The irregularity of the injury is especially noted in the sections from the jejunum.

Ileum shows extensive injury and destruction of villous epithelium. The epithelium covering the villi and crypts has disappeared except for some rapidly growing epithelial cells apparently regenerating in an effort to cover the denuded areas. Polymorphonuclear and mononuclear leucocytes are rather numerous at the bases of the crypts.

Colon shows a certain amount of mucoid degeneration and here and there an occasional focus of polymorphonuclear infiltration especially under the areas where the epithelium is injured.

Dog 19–101. Clinical History and Autopsy (See Table III).

Feb. 17. Dog is active and in good condition.

Feb. 21. ¼ gr. of morphia is given 1 hour previous to the radiation; 117 milliampere minutes of radiation are given with a current strength of 7.5 milliamperes, at an E. M. F. of 103 kilovolts. It was then found necessary to give the remaining 233 milliampere minutes at a current strength of 10 milliamperes with an E. M. F. of 90 kilovolts. 2 mm. of aluminum filter used, spark-gap of 9 inches between sharp points. The skin-target distance is 10 inches.

Apr. 7. Dog is exposed to 450 milliampere minutes of radiation over the abdomen and sacrificed under ether anesthesia on Apr. 11.

TABLE III.

Roentgen Radiation.

350 Milliampere Minutes over Thorax Negative.

Dog 19–101.

Date.	Weight.	White blood corpuscles per c. mm.	Urine.		Diet.	Remarks.
			Volume.	Total N.		
	lbs.		*cc.*	*gm.*		
Feb. 17	34.0	8,000	—	—	Water.	Dog normal.
" 20	31.0	—	—	—		
" 21	30.4	7,800	400	3.03		
Feb. 21	X-rays, 350 milliampere minutes, over thorax. Abdomen shielded.					
Feb. 22	30.1	7,300	415	3.02	Water.	Dog normal.
" 23	30.0	7,900	605	2.86	"	
" 24	29.1	5,600	565	2.69	"	
" 25	28.8	7,300	605	2.52	"	
" 26	28.1	7,600	408	2.49	"	
" 27	28.1	7,400	370	2.24	"	Dog normal.
" 28	27.8	6,900	335	2.21	"	
	Experiment ended.					
Apr. 11	31.3	7,600	—	—		
	Ether anesthesia and autopsy at once.					

Autopsy.—The pleural and pericardial surfaces are moist and shiny. The heart and lungs are negative. The other changes do not concern the experiment given in Table III.

Dog 19–50. Clinical History and Autopsy.

To be contrasted with Table III; a control experiment over the abdomen.

Feb. 10. Dog active and normal; fasting.

Feb. 11. ¼ gr. of morphia 1 hour previous to radiation; 340 milliampere minutes are spread over the abdomen in the usual seven areas; screening of the thorax and lower pelvis with lead-foil. A current strength of 7.5 milliamperes at 97 kilovolts is backed up by a 9½ inch spark-gap; 2 mm. of aluminum filter the radiation. The skin-target distance is 10 inches.

Feb. 12. The dog is lively and active. A small amount of white frothy vomitus and some fluid brown feces appear on this day.

Feb. 13. There is evidence of slight intoxication. The pulse tension is low. The vomitus is of large volume, as much as a pint in an hour, and is deeply bile-stained. The diarrhea is frequent but scanty in amount and consists mostly of blood. At times, it is black, thick, and tarry. The dog is quite weak.

Feb. 14. The intoxication is quite severe. The animal is weak. The pulse is of low tension and quite irregular. The vomitus is large in amount and deeply bile-stained. The diarrhea is profuse and frequent. At times, the fecal material seems to be made up practically of pure blood and contains many clots. Again it may be tarry and vile smelling. The dog is comatose and is sacrificed under ether anesthesia. Autopsy at once.

Autopsy.—Thoracic viscera and serous surfaces are negative. The liver, spleen, adrenals, kidneys, lymph nodes, and genitourinary tract are negative. The pancreas shows a few small fat necroses.

Stomach: filled with a bloody mucous material; mucosa pale and normal.

Small intestine is spastic and contains a small amount of bloody fluid. The mucosa is a dull red, darker in the duodenum, lighter in the ileum. Longitudinal folds with hemorrhagic crests are evident in some sections, especially the paler sections of the lower jejunum, but they are masked in the duodenum by the intense dark dull red of the mucosa there. The intestinal wall is thin, apparently due to much loss of substance in the mucosa. This is especially marked in the ileum.

Colon and *cecum* contain a bloody fluid. Their mucosa is in general pale

Histological Sections.

Liver, kidneys, adrenals, uterus are normal. Spleen shows some pulp atrophy and prominence of the reticulum Pancreas contains a few fat necroses in one section, otherwise it is normal. In the ovary, the chromatin network of the ova is broken up, though the nucleoli are still present. The ova in general appear abnormal and seem to be degenerating.

Stomach and *colon* are normal.

Small intestine presents the usual picture of necrosis and destruction of villous and crypt epithelium, regeneration of epithelium with mitotic figures, and infiltration of polymorphonuclears into the bases of the crypts.

In the *ileum* the villi show only a naked reticulum with a few groups of regenerating epithelial cells and the lymph follicles. The blood vessels in the mucosa are frequently thrombosed.

The *mesenteric lymph nodes* are filled with red blood cells and polymorphonuclears and phagocytic cells containing cell debris and pigment.

TABLE IV.

Roentgen Radiation.
Sublethal Dose over Abdomen.

Dog 19–85.

Date.	Weight. *lbs.*	White blood corpuscles per c. mm.	Diet.	Remarks.
Feb. 4	25.0	8,000	Water.	Dog normal.
Feb. 4	X-rays, 300 milliampere minutes, over abdomen. Thorax shielded.			
Feb. 5	24.5	6,000	Water.	Dog normal.
" 6	24.0	8,000	"	
" 7	23.5	3,400	"	Inactive.
" 8	23.3	8,400	"	Vomitus and diarrhea.
" 9	23.0	5,800	"	No diarrhea or vomitus.
" 10	23.0	7,800	"	Clinical improvement.
" 11	22.5	5,400	Mixed.	
" 20	25.0	7,400	"	Good condition.

Dog 19–85. Clinical History (See Table IV).

Feb. 3. Dog active and in good condition; fasting.

Feb. 4. ½ gr. of morphia given 1 hour before radiation. The upper part of the body above the level of the xiphisternum is screened by 2 mm. of sheet leadfoil. Exposures made over the abdomen and pelvis in seven parts. The current strength is 7.5 milliamperes at an E. M. F. of 100 kilovolts, backed up by a 9½ inch spark-gap. The radiation is filtered by 2 mm. of aluminum. The skin-target distance is 10 inches. The total exposure time is 40 minutes—300 milliampere minutes. The dog is clinically unaffected by the exposure to radiation.

Feb. 5 to 7. Dog normal.

Feb. 8. The animal shows a slight degree of intoxication. The vomitus which appears is mostly a clear frothy mucus. Diarrhea is brownish and scanty.

Feb. 9. Mild intoxication; no diarrhea or vomitus.

Feb. 10. From this date, recovery to normal is rapid.

DISCUSSION.

At the very start of this discussion, we wish to point out the fact that these experiments concern normal dogs and not human beings suffering from disease, for example leucemia, cancer, and so forth. We feel that these experiments are less complicated for this very reason and may give us the opportunity to correct mistaken impressions gotten from the complex reactions which may result from Roentgen radiation of a diseased human being.

Our negative results of thorax radiation (up to 512 milliampere minutes) are of interest in ruling out convincingly certain of the body cells which have been suspected as causative factors in the general Roentgen ray intoxication. For example, these enormous doses given over the normal thorax must injure more bone marrow cells than do the abdomen exposures. The ribs and thoracic vertebræ surely contain more units of red marrow than do the lumbar vertebræ. Examination of the rib marrow some days after an exposure shows almost complete disappearance of red marrow cells, so we know that this reaction has taken place, yet there is not a trace of any clinical disturbance. This rules out the red marrow as a factor in the characteristic systemic disturbance noted in our experiments.

These same arguments apply to lymphatic tissue. The lymph glands in thorax and abdomen usually show some loss of cells but not to any very great extent. It is significant that Peyer's patches in the intestine may show only moderate injury when the epithelium covering the lymph follicles and lining the crypts has completely vanished due to the x-ray injury. In our experiments, therefore, the intestinal epithelium is much more sensitive to injury than are the cell elements of the lymph gland and Peyer's patch.

The spleen of the dog is not profoundly affected by the Roentgen radiation. For one thing, it is not as cellular as the human spleen, but we feel that the normal spleen cells are not easily destroyed by the x-rays. After large exposures we note a moderate atrophy of the spleen with shrinkage of the Malpighian corpuscles, but plenty of lymphocytes remain even after intensive radiation.

The thorax exposures, of course, cover the heart and lungs, which may be dismissed without comment. This area also includes a large part of the *circulating blood.* We conclude, therefore, that the x-rays have no injurious effect upon the circulating blood cells.

The thorax exposures therefore rule out several factors which at one time or another have been thought to be responsible for the general systemic intoxication red bone marrow, lymphatic tissue, and circulating blood. We may now consider the various tissues in the abdomen which may be responsible for the fatal reaction following large doses of the x-rays.

The kidneys are very slightly disturbed, as is shown in a paper of this series (McQuarrie and Whipple (4)).

The liver is not concerned and we believe can be ruled out without question. There is no histological evidence of injury, but more than that, there is no evidence of functional disturbance. Dogs with bile fistulæ show no disturbance in function and no change in the level of bile acid and bile pigment output.[1]

The pancreas and adrenals show no histological evidence of injury by the x-rays. We know of no functional disturbance which may be attributed to these structures.

The gastrointestinal tract is remarkably sensitive to the Roentgen rays. In subsequent experiments, we shall review the remarkable ulcers which can be produced in the stomach, but as a whole, the stomach and colon are considerably more resistant than the small intestine.

The *epithelium of the small intestine* from the pylorus to the ileocecal valve is remarkably sensitive to the Roentgen rays. These doses used will cause necrosis of this epithelium with practically complete disappearance. We believe the symptoms of intoxication and the final lethal poisoning are due to this injury of the intestinal epithelium. We need not refer to the severe intoxication which develops following a functional disturbance of this same epithelium (intestinal obstruction). We may infer that the x-rays may produce sublethal injury to this intestinal epithelium which may be responsible for a variety of disturbances associated with gastrointestinal symptoms.

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[1] Experiments to be published elsewhere.

SUMMARY.

Roentgen radiation of the thorax (abdomen shielded) in dogs, even with large doses (up to 512 milliampere minutes), gives no clinical evidence of intoxication. There may be a transient leucopenia and a slight rise in urinary nitrogen.

Roentgen radiation of the abdomen (thorax shielded) in dogs, with a dose of 350 milliampere minutes, will almost certainly cause a fatal intoxication. Smaller doses may be survived but usually with signs of gastrointestinal intoxication.

This lethal intoxication due to abdominal radiation presents a remarkably uniform clinical and anatomical picture. There is a latent period of 24 to 36 hours, during which the dog is perfectly normal clinically. The 2nd day usually shows the beginning of diarrhea and perhaps some vomitus. The 3rd and 4th days show progressive intoxication with increasing vomiting and bloody diarrhea until the dog becomes stuporous. Death is almost always on the 4th day.

Anatomically the only lesions of significance are to be found in the small intestine. The epithelium of the crypts and villi shows more or less complete necrosis, and this condition may involve almost all of the small intestine. The epithelium may vanish completely except for a few cells here and there which have escaped and are often found in mitosis, probably an effort at repair and regeneration.

We are forced to the conclusion that this remarkable injury of the epithelium of the small intestine is responsible for the various abnormal reactions and final lethal intoxication which follow a unit dose of Roentgen radiation over the abdomen of a normal dog. This sensitiveness of the intestinal epithelium to x-rays is not appreciated and should be given proper consideration in clinical work.

In conclusion we wish to express our sincere appreciation for the assistance and advice given us by members of the Department of Roentgenology of the University of California Hospital—Drs. Howard E. Ruggles, Lloyd Bryan, Orrin S. Cook, and Elizabeth Schulze.

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[Reprinted from The Journal of Experimental Medicine, February 1, 1922,
Vol. xxxv, No. 2, pp. 203–211.]

ROENTGEN RAY INTOXICATION.

II. A Study of the Sequence of Clinical, Anatomical, and Histological Changes Following a Unit Dose of X-Rays.

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(Received for publication, August 16, 1921.)

The experimental data given below supplement that of the first paper of this series. All these experiments make it very clear that the initial injury of the epithelium of the small intestine is responsible for the severe clinical reaction and lethal intoxication. The fact that this epithelium is sensitive to the Roentgen rays has not hitherto been clearly recognized. For this reason, it seemed desirable to study carefully this reaction and record the histological and gross changes which follow day by day after the initial exposure.

It is well to recall that the urinary nitrogen in these experiments shows a prompt increase on the day following the exposure (experiments in Paper I and those of Hall and Whipple (2)). This increase is not great and no considerable rise is usually noted until the last day before death. This urinary nitrogen curve does not in any way parallel the clinical reaction, which usually presents a latent period of 24 to 48 hours. It is of great interest to note below that within the first 24 hours, there are distinct histological abnormalities in the epithelium of the small intestine, yet this is always a period free from any clinical evidence of injury but showing a slight rise in urinary nitrogen.

The appearance of clinical intoxication coincides with the actual initial cell disintegration which is so evident in the epithelium of the small intestine in the second 24 hours. It is of interest that the crypt epithelium suffers first and shows evidence of actual necrosis and solution before any change is apparent in the epithelium covering the villi. This may well indicate some undetermined differences in function as well as in histology between these two types of epithelial cell. The epithelium of the villi does not show much disturbance until the 3rd day, when the clinical intoxication is also well marked with bloody diarrhea and bile-stained vomitus.

The severity of the intoxication on the 3rd and 4th days coincides with the disappearance of much of the epithelium all through the small intestine. One may choose to explain this reaction as due to absorption of toxic substances and *invasion of bacteria from the lumen of the injured intestine.* These factors may be in part responsible for the intoxication, but we believe there are other possibilities which must be considered. Some of these are to be reviewed more in detail in subsequent papers of this series.

EXPERIMENTAL OBSERVATIONS.

All materials used in this study are obtained from dogs killed under anesthesia and autopsied at once. Tissues are fixed at once in formaldehyde (10 per cent) and stained usually by the routine hematoxylin-eosin method. Control tissues from normal dogs are fixed and prepared in exactly the same manner. A minimum of mechanical handling before fixation is necessary in this work. Routine examination of all organs in gross and histologically is carefully performed, but in the protocols normal findings are often omitted from the record.

Experiment 1. Lethal Dose of Roentgen Rays over Abdomen. Killed after 2 Hours.

Dog 20-33.—Normal, mongrel, adult, male; weight 32.5 pounds. Dog fasting for 4 days previous to radiation. Roentgen ray exposure over abdomen in four quadrants; no screens used, but usual 2 mm. aluminum filter. In all, 480 milliampere minutes given; E. M. F. of 89 kilovolts; current strength 8 milliamperes; skin-target distance of 10 inches. Exposure given between 3 and 5 p. m. 6 p. m. Dog sacrificed by bleeding under ether anesthesia.

Autopsy.—Performed at once. All viscera examined carefully and found to be normal, except for the kidneys, which show a few scars in the cortex. The intestinal tract shows no congestion and no abnormalities of any sort.

Histological Sections.—All organs and tissues examined in routine sections. There are very few abnormalities. The kidneys show arteriosclerosis and dense scars; the prostate shows some hyperplasia. Spleen and mesenteric glands show a good deal of nuclear fragmentation in centers of follicles.

Gastrointestinal tract at first sight is negative, but careful comparison with sections of normal control dogs convinces one that some of the nuclei in the crypt epithelium of the duodenum and jejunum do show slight abnormalities. These nuclei are fuzzy looking and the chromatin is beginning to show very slight evidence of injury. These changes are much more noticeable in the lymph glands of this region.

Experiment 2. Lethal Dose of Roentgen Rays over Abdomen. Killed after 24 Hours.

Dog 19-130.—Apr. 15, 2 p.m. Normal, old adult, male mongrel; weight 45 pounds. Dog fasting for 3 days previous to radiation. X-ray exposure over abdomen, 450 milliampere minutes; 90 kilovolts; current strength 7.5 milliamperes; spark-gap 9 inches; 2 mm. aluminum filter; skin-target distance 10 inches. Dog given morphia ($\frac{1}{2}$ gr.) before exposure.

Apr. 16, 2 p. m. Dog normal in all respects; chloroform anesthesia and exsanguination.

Autopsy.—Done at once. All viscera examined with care and are normal with the following exceptions: *Small intestine* is tightly contracted and contains only bile-stained fluid. Duodenum and jejunum show the mucosa thrown up into longitudinal folds whose crests are specked with ecchymoses. There are a few small scattered ecchymoses all through the small intestine. The mucosa is not markedly congested but is unusually *bile-stained* and this may be due to actual vital staining of the injured cells.

Histological Sections.—Spleen and mesenteric lymph nodes show cell necrosis in lymph follicles and numbers of phagocytes. All of small intestine shows definite nuclear changes in the epithelium of the crypts. Nuclei are pale or show fragmented chromatin material. There is a definite invasion of polymorphonuclear leucocytes. The epithelium of the tips of the villi shows no distinct change as yet.

Experiment 3. Lethal Dose of Roentgen Rays over Abdomen. Killed after 48 Hours.

Dog 19-101.—Apr. 9. Normal, adult, female mongrel; weight 33 pounds. Dog fasting for 4 days and through experiment. X-ray exposure over abdomen, 450 milliampere minutes; 90 kilovolts; current strength 7.5 milliamperes; spark-gap 9 inches; 2 mm. aluminum filter; skin-target distance 10 inches. Dog given morphia ($\frac{1}{2}$ gr.) before exposure. Thorax and pelvis screened by lead.

Apr. 10. Dog is normal.

Apr. 11. Dog bright and active, but vomits some bile-stained mucus at 2 p. m. Ether anesthesia and sacrifice at 4 p. m.

Autopsy.—At once. All organs examined with care and are normal except where noted otherwise. Stomach contains a little bile-stained mucus, but mucosa is normal. Small intestine contains a little bile-stained mucus and a little bright red blood in one section; the mucosa in general is pale, but shows scattered ecchymoses. There are longitudinal folds whose crests are pink and dotted with ecchymoses. There are few congested patches in the ileum. Colon is spastic but mucosa normal.

Histological Sections.—Stomach mucosa is normal. There is a sharp line of transition from normal stomach to abnormal duodenal mucosa. Here the duodenal crypts show fragmented nuclei and cell degeneration. A few wandering cells are in evidence. Mitotic figures indicate an effort on the part of the epithelium to regenerate. There is some edema of the submucous tissues. The epithelium covering the villi appears uninjured. This condition is general throughout the small intestine. There is a sharp line of demarcation at the ileocecal valve and the colon mucosa seems practically normal. Colon shows occasional crypts presenting degenerating epithelial cells and invasion by wandering cells. Ovary shows degenerating ova with chromatin fragmentation.

Experiment 4. Lethal Dose of Roentgen Rays over Abdomen. Killed after 3 Days.

Dog 19–50.—Feb. 11. Normal, adult, female spaniel; weight 32 pounds. Dog fasting for 2 days before radiation and through experiment. X-ray exposure over abdomen, 340 milliampere minutes; 97 kilovolts; current strength 7.5 milliamperes; spark-gap 9½ inches; 2 mm. aluminum filter; skin-target distance 10 inches. Thorax and pelvis shielded by lead. Morphia (¼ gr.) before exposure.

Feb. 12. Dog lively; a little frothy vomitus in afternoon.

Feb. 13. Dog less active; blood-stained stools and bile-stained vomitus.

Feb. 14. Dog weak, much bright blood in stools, which contain mucus and clots, very foul smelling; vomiting less frequent. Ether anesthesia and sacrifice.

Autopsy.—At once. Viscera all examined carefully and found normal unless otherwise noted. Kidney: slight scarring of cortex. Pancreas: a few tiny fat necroses close to bile duct. Stomach contains blood-tinged, bile-stained mucus, but its mucosa seems normal. Small intestine contains a little bloody mucus and it is irregularly contracted. *Duodenum* shows a dark, glassy, red mucosa which is thin and in places wrinkled. The mucosa has lost its velvety appearance. This is explained by the histology. The rest of the small intestine shows much the same picture but the surface is mottled and some areas are much more abnormal than others. Peyer's patches are rather conspicuous, due to the thinning of the mucosa. This change in the mucosa begins sharply at the pylorus and ends as sharply at the ileocecal valve. Colon: mucosa is in general pale but there are a few ecchymoses.

Histological Sections.—Unless noted are negative. Lymph follicles in mesenteric glands and spleen show evidence of injury and removal of damaged cells. Ovary shows degenerating and necrotic ova. Bone marrow—lumbar vertebra—shows few marrow cells remaining and much cell debris in large phagocytes. Stomach: some cells in the mucosa appear slightly abnormal but there is nothing definite. *Duodenum:* much of the epithelium covering the villi and lining the crypts has disappeared. In many places, the villi appear as naked polyps, a little swollen and containing dilated capillaries. The crypts are often filled with cell debris and wandering cells. At times mitotic figures are seen in the remaining epithelium. Wandering cells are conspicuous in all this area—usually polymorphonuclears. There is a noticeable edema of the submucosa. The whole structure of the mucosa has collapsed because of the destruction of the epithelium. Some sections show a mucosa which is almost normal. This "escape" of these areas will be discussed later. *Jejunum* and *ileum* show the same extensive destruction of the epithelium with consequent collapse of the familiar structure of the mucosa. Many sections would scarcely be recognized as coming from the small intestine. In places the mucous surface is covered with an exudate of red and white cells with fibrin. Some areas show only a moderate injury, corresponding to the gross appearance.

Experiment 5. Lethal Dose of Roentgen Rays over Abdomen. Killed after 4 Days.

Dog 19–80.—Jan. 21. Adult, female mongrel; weight 20.5 pounds. Dog fasting for 2 days previous to radiation and subsequently. X-ray exposure over abdomen, 350 milliampere minutes; 95 kilovolts; current strength 7.5 milliamperes; spark-gap 9½ inches; skin-target distance 10 inches; 2 mm. aluminum filter. Dog given morphia (¼ gr.) before radiation. Thorax and pelvis screened with lead.

Jan. 22. Dog active and normal.

Jan. 23. Dog less active, but no other clinical symptoms.

Jan. 24. Dog sick with soft stools and usual vomitus.

Jan. 25. Dog prostrated; stools contain fresh blood and clots; temperature is falling and dog becoming comatose; ether anesthesia and sacrifice 90 hours after radiation.

Autopsy.—At once. Unless otherwise stated viscera are normal. Mesenteric glands are enlarged, moist, and congested. Stomach contains blood-tinged mucus and its mucosa is normal. Small intestine contains a little bloody fluid and a few worms. The mucosa in many loops is dark red and glassy, specked with ecchymoses. Other areas are more nearly normal and show the velvety texture of the mucosa. The jejunum seems to be less uniformly injured than the duodenum and ileum. Longitudinal folds with hemorrhagic crests are found in all parts of the small intestine. Colon shows a pale mucosa specked with occasional ecchymoses.

Histological Sections.—The changes noted in this autopsy are almost identical with those described in detail in Experiment 4. The epithelial cells of the mucosa are conspicuous by their absence, and there is more evidence of mitosis and early repair on the part of the few epithelial cells which remain in the injured crypts. There is a moderate grade of edema of the submucosa and some invasion by wandering cells. The congested capillaries in the naked villi are the obvious source of the blood in the intestinal contents. Occasional thrombi are noted in these vessels. Stomach and colon show minimal changes. In similar experiments one may find considerable epithelial degeneration in the crypts of the colon mucosa but it is obvious that this epithelium is much more resistant than is that of the ileum.

DISCUSSION.

We may review the positive features of this Roentgen ray reaction in dogs day by day following the exposure. The first 24 hours show no clinical disturbance. These dogs would pass as normal on the most critical examination. The urinary nitrogen shows a slight but constant increase above normal. The body cells, however, show some definite changes. The spleen and lymph glands show readily recognized cell injury and nuclear fragmentation within 2 hours. The epithelium of the crypts of the small intestine also within 2 hours shows distinct but faint nuclear changes. Within 24 hours, this intestinal epithelium shows nuclear fragmentation and cell injury together with a wandering cell reaction (leucocytes and macrophages). The ova in the ovary show the familiar nuclear reaction of degeneration.

After 2 hours, the small intestine may show a distinct flushing of the mucosa. This may or may not be due wholly to the x-rays, but it may be noted as perhaps similar to the erythema of the skin which may be noted a few hours after an erythema skin dose. The skin may not show distinct injury (burn) for many days after this initial reaction to injury by the x-rays. Apparently the various body cells have individual peculiarities in their reaction to toxic doses of the x-rays, especially as regards the latent period. Compare leucocytes, intestinal epithelium, and skin epithelium.

Within the second 24 hours after a toxic dose of the x-rays, we see many distinct changes from normal. Usually there is definite clinical abnormality, although at times this may be very slight. As a rule, there is loss of appetite and activity. The dog often vomits a little mucus and passes a soft stool or two. Autopsy at this time shows an intestinal tract which may appear normal except for a few ecchymoses in the small intestine. There may be abnormal bile staining of the mucosa, due in part perhaps to the presence of dead epithelium. The intestine in general is apt to be spastic and reacts to the stimuli with very vigorous contractions. This irritation of the muscle coats is probably the result of the early inflammation beginning in the mucosa and submucosa. This spastic tendency, we believe, explains the longitudinal folds which are so conspicuous in the 3rd and 4th days with their crests marked out by congestion and hemorrhage.

Histologically, in the second 24 hours, we find a number of important changes, practically all in the small intestine. The epithelium of the crypts shows severe injury with nuclear destruction and even cell necrosis and desquamation, so that the crypt lumina are filled with cell debris and wandering cells. The epithelium of the villi still remains to all intents normal. The wandering cells are not yet conspicuous in the submucosa and are usually polymorphonuclears. There are a little submucous edema and occasional extravasation of red cells. These changes are conspicuous from the pylorus to the ileocecal valve. The colon shows occasional small patches in which the crypts show similar but less advanced injury.

The third 24 hour period is one of violent clinical disturbance and frank anatomical changes, mostly limited to the small intestine. The dog is constantly retching and frequently vomiting a thick mucoid bile-stained material. There is usually much fluid diarrhea, often with blood and mucus. Tenesmus may be much in evidence. Smears from fecal material show numbers of red and white blood cells, epithelial cells, and bacteria. This period gives marked anatomical changes in a typical case. The entire small intestine from pylorus to ileocecal valve looks raw, red, and thinned out. Some areas may escape and appear relatively normal. We believe these areas of severe injury of the mucosa are to be explained by overlapping areas of exposure. They may also be in the uppermost coils of intestine in the abdomen.

The histological material is of great interest but we cannot review the details. There is almost complete loss of crypt and villous epithelium, and the mucosa collapses into an atypical matrix of villi and empty crypts. Some sections are almost unrecognizable. Villi appear as naked polyps with congested capillaries. A few crypts may contain occasional epithelial cells which are large and contain mitotic figures—an attempt at early repair. The bloody fecal contents are easily explained by this picture. In spite of these raw areas we do not observe the intense inflammatory reaction which might be expected. There are a little edema and a distinct increase in wandering cells. We believe this point is of much interest as regards the common conception of the intestinal epithelium as the sole barrier between the intestinal bacteria and the blood and lymph stream. This point will be taken up again in detail with a study of its bacteriology.

The fourth 24 hour period is much like the third but shows increasing intoxication. Death usually follows on this day. There is great loss of weight and strength. Vomitus and diarrhea continue unabated and the dog finally goes into coma preceding death. The histology is much like that described for the 3rd day, but evidences of epithelial repair may be more conspicuous and of fascinating interest to the histologist.

It is a common observation that lymph follicles and Peyer's patches show less injury than do the neighboring epithelial cells of the mucosa. Our sections would indicate that the epithelium of the small intestine is at least as sensitive to the x-rays as are the lymphocytes of the same area. In fact, by a large dose of the x-rays, we can actually destroy all of the epithelium, while many clusters of lymphocytes still remain. It is possible, of course, that lymphocytes in different parts of the body have varying susceptibilities to radiation, so that this observation in the intestine may not be applicable to the lymphocytes elsewhere in the body.

It may be noted at this time that a recent publication of Denis, Martin, and Aldrich (1) differs considerably in its interpretation of the abnormalities noted after long x-ray exposures. Rabbits were used and in our experience are not as favorable animals for this work, although the changes are similar In their discussion, Denis, Martin, and Aldrich suggest that these epithelial changes in the intestine are "the result rather than the cause of the intoxication" and not primarily responsible for the intoxication. We are confident that our experiments give convincing proof that this epithelial injury in the small intestine is due to the x-rays and is in itself responsible for the progressive and lethal intoxication.

SUMMARY.

Roentgen radiation in lethal dosage, given over the abdomen of a normal dog, is followed by a physiological reaction of remarkable uniformity.

The first 24 hour period following the exposure is negative clinically and anatomically, but histologically we see frank changes in the bone marrow, spleen, lymph glands, and ovaries. There are definite nuclear changes with degeneration in the crypt epithelium of the small intestine.

The second 24 hour period shows slight clinical disturbances of gastrointestinal nature (vomitus and diarrhea). The mucosa of the small intestine shows scattered ecchymoses, but the histology of the small intestine is important. The necrosis of the crypt epithelium may be almost complete, while the epithelium of the villi remains practically intact. There are a little edema and invasion of wandering cells.

The third 24 hour period shows increasing clinical disturbance with vomiting and bloody diarrhea. Anatomically the small intestine from the edge of the pylorus to the rim of the ileocecal valve looks raw, red, and inflamed. The crypt and villous epithelium has in large part vanished, leaving a collapsed framework of the mucosa showing a little edema and invasion of wandering cells.

The 4th day marks the peak of the intoxication, and death usually takes place at this time, preceded by coma. Anatomically and histologically the picture is like that of the 3rd day. There is more evidence of mitosis and efforts of repair on the part of the intestinal epithelium.

The stomach is not concerned in this reaction, but the colon may show evidences of a slight injury. The colon is obviously much more resistant than is the small intestine.

We believe the evidence is conclusive that the injury done the epithelium of the small intestine is wholly responsible for the stormy clinical picture and fatal intoxication.

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[Reprinted from The Journal of Experimental Medicine, February 1, 1922,
Vol. xxxv, No. 2, pp. 213–224.]

ROENTGEN RAY INTOXICATION.

III. Speed of Autolysis of Various Body Tissues after Lethal X-Ray Exposures.

The Remarkable Disturbance in the Epithelium of the Small Intestine.

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(Received for publication, August 16, 1921.)

The question of Roentgen ray injury and certain modifications of intracellular ferments has been reviewed by Hall and Whipple (1). We must admit that the various methods of studying intracellular ferments are at present very crude and probably give mere hints as to what goes on within the cell. These ferments are accustomed to their normal environment which is very carefully balanced by the presence of the circulating fluids which remove waste products and furnish necessary supplies to the cells. The reaction of these ferments in a mass of dead or dying cells cut off from circulatory influences may or may not have any significance as to the true physiological function of these ferments.

We have submitted much evidence of true cell injury involving both nucleus and protoplasm following x-ray exposures. We submit below evidence to show that cell autolysis in the epithelium of the small intestine is profoundly modified by x-ray exposures. Some investigators go so far as to claim that primary injury of cell ferments is a fact and is in itself responsible for subsequent reactions and cell death. But how can one tell that this modification of cell autolysis is due to a primary ferment influence or some other physiological disturbance which influences the ferments secondarily? When the investigator gives this explanation of primary cell ferment reaction, he is indeed in distress when he attempts to explain the great differences in the effect of the x-rays on different cell structures. Our methods permit of a distinction in cell and nuclear structure between these cells which react so very differently to the x-rays. But present methods do not permit us to recognize a great multitude of individual cell ferments in the parenchymatous organ cells. Compare the cells of the bone marrow, intestinal mucosa, pancreas, and liver. They are all rich in ferments but they react very differently indeed to the x-rays. One must be very conservative in the analysis of data concerning intracellular ferments and deductions drawn from such experiments.

It was pointed out by Hall and Whipple (1) that the intestinal epithelium in radiated dogs was subject to rapid postmortem autolysis, so much so, that a few hours after the death of the radiated animal very little of the epithelium could be demonstrated. These workers also indicated that the crypt epithelium was more abnormal in this respect than the tip epithelium. With these observations in mind, a series of autolysis experiments was undertaken using sections of organs from the series of dogs described in Paper II. In this series an attempt is made to demonstrate the various changes in cell autolysis observed at various periods following a lethal x-ray exposure.

Method.

A series of dogs was exposed to 300 to 480 milliampere minutes of radiation diffusely over the abdomen. These experiments are described in the preceding paper. Dogs were sacrificed under chloroform or ether anesthesia at various periods of 2, 24, 48, 72, or 96 hours after exposure to radiation. A set of sections of each organ for control is fixed immediately in 10 per cent formaldehyde. Five sets of sections, each section about 3 to 5 gm. in weight, are cut from each organ immediately after death of the animal. Each section is placed, while still warm, in a bottle containing approximately 150 cc. of warm 0.9 per cent sodium chloride solution previously saturated while warm by shaking for an hour with an excess of chloroform. All the bottles are then warmed to 37°C., corked, placed in an incubator at 37°C., and the tissues allowed to autolyze. A set of sections is removed at 4, 8, 10, 12, and 24 hours respectively and immediately fixed in 10 per cent formaldehyde. There is no putrefactive odor present in the autolysis bottles, even after 24 hours autolysis of intestinal sections, indicating that the growth of putrefactive bacteria has been inhibited by the chloroform and suggesting that the autolysis is due to the endogenous ferments. There are no free chloroform globules present in the autolysis bottles, it being completely held in solution. The odor of chloroform is still quite strong after 24 hours autolysis. Several sets of experiments were done with sodium chloride solution without chloroform, but these were found to be very irregular, probably due to variation in the outgrowth of bacteria.

Organs from three normal unradiated dogs are treated to the same routine and used as controls.

Histological Method.

The autolysis sections are studied with regard to the effect of radiation upon the rapidity of autolysis *in vitro*. Gradations of autolysis are based upon the intensity of staining of the nuclei, the integrity of the cell outline and the cell contents, and the complete digestion of the cell itself. In designating changes for the purposes of constructing curves indicating the speed of autolysis, the following criteria are used:

0 means no autolysis perceptible.

A or + means cell nuclei are pale and washed out, as compared with the control of that organ—slight autolysis.

B or ++ means nuclear structure is lost, run together, or indistinct—well advanced autolysis.

C or +++ means the nuclear outline is shadowy and indistinct. There may appear to be nothing left but nuclear debris in the space occupied by the cell—marked autolysis.

D or T means total autolysis; the nuclei are gone, though the basement membranes are usually intact. At this stage, there is nothing left but pale fibroblasts, polymorphonuclears, endothelial cells of the blood vessels, and the fibrillar intracellular matrix.

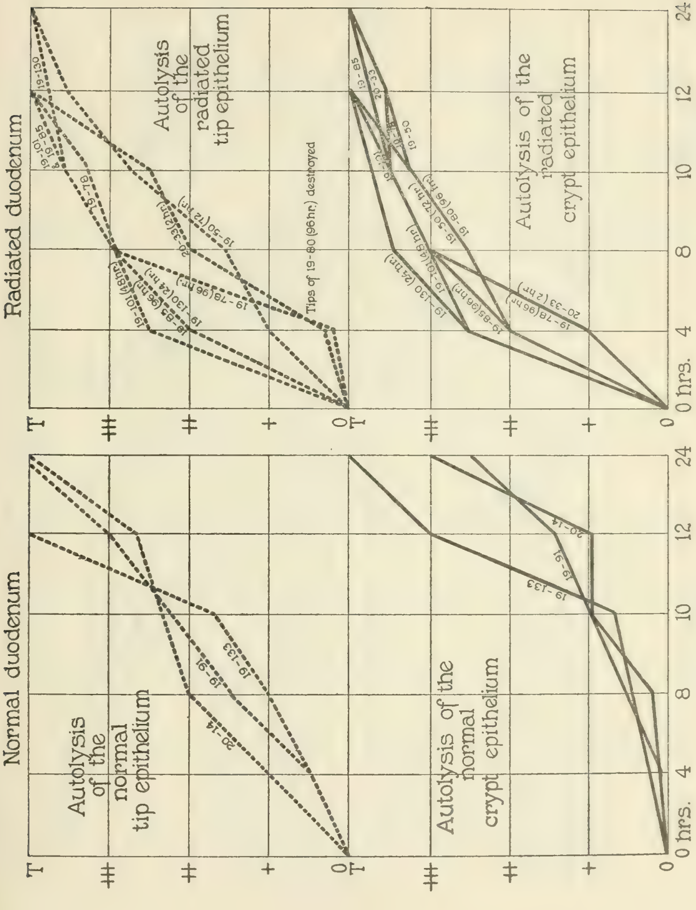
It would seem that these arbitrary periods of autolysis are irregular in sequence but these periods were established by preliminary experiments and seemed to us to give the required information. In plotting these findings, it also serves our purpose to consider the individual stages (or extent) of autolysis and the individual period of autolysis as equal and numerically progressing units upon the coordinates. It is evident that if all of the constants are equal and presented in the same manner, the experimental results are being compared upon a satisfactory basis. Therefore, although all of our units are arbitrary, they are constant throughout and any consistent variation in the plotted curves may be considered as demonstrating changes produced by the experimental procedure (exposure to radiation).

EXPERIMENTAL OBSERVATIONS.

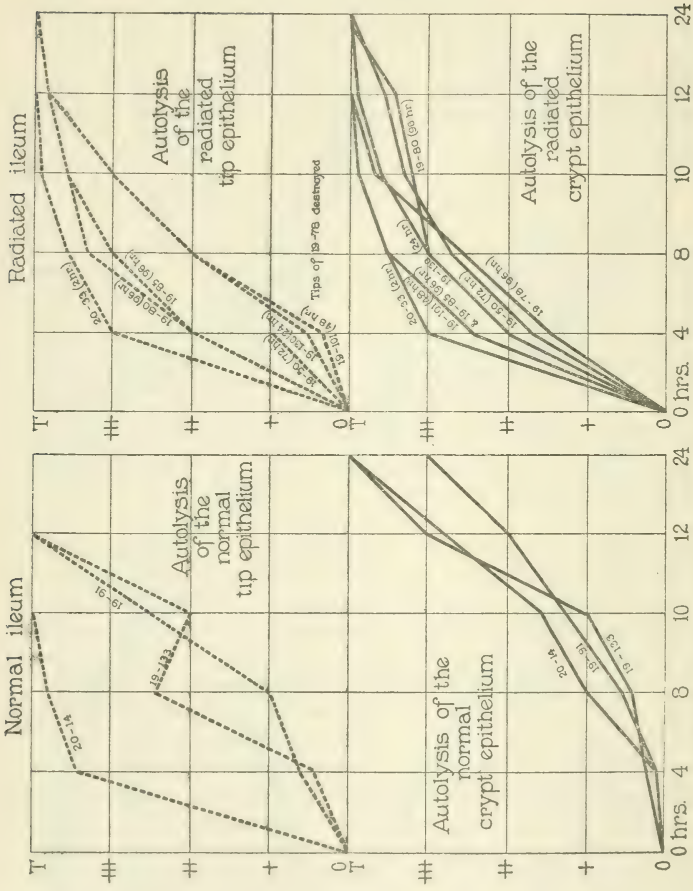
All the histological material for this study was fixed in 10 per cent formaldehyde and stained by the usual hematoxylin-eosin method, using paraffin sections. About 600 slides were studied and reviewed at least three separate times as unknown specimens to prevent any bias from knowledge of sequence of events in autolysis periods. The grades of autolysis have been established and described in the preceding paragraph. Curves of the various tissue reactions were established in form similar to Text-figs. 1 and 2. These many charts cannot be published but the statements made below are based on a study of such charts and sections. Much of the more important data can be studied in Table I.

We may note that certain cells common to organs and tissues are not much, if at all, disturbed by these long x-ray exposures. Such are fibroblasts and mature connective tissue cells, endothelial cells and macrophages, muscle tissues, and certain white blood cells. All these tissues are resistant to autolysis in the control and radiated animals and may appear as very little changed even by the long 24 hour period of autolysis at 37°C. Some, but not all, of these cells are supposed to be poor in ferments.

The more specialized cell types of the abdominal viscera are our chief concern. In the gastrointestinal tract, we differentiate between the tip epithelium which covers the villus and the crypt epithelium which is more of the secreting cell type. In the spleen and mesenteric lymph nodes, we have made a rather broad distinction between the pulp and the Malpighian bodies; in the pancreas between the isles of Langerhans and the remainder of the parenchyma. In the kidney, the proximal convoluted tubules are contrasted with the straight



TEXT-FIG. 1. Comparison of the autolysis of the duodenum of normal and of radiated dogs.



TEXT-FIG. 2. Comparison of the autolysis of the ileum of normal and of radiated dogs.

TABLE I.

Autolysis during Periods of Hours as Indicated.

	Control.					2 hrs. after x-rays.					24 hrs. after x-rays.					48 hrs. after x-rays.					72 hrs. after x-rays.				
Hrs. autolysis.	4	8	10	12	24	4	8	10	12	24	4	8	10	12	24	4	8	10	12	24	4	8	10	12	24
Duodenum crypt.	0	0	A	A	C	A	C	C	D	—	B	C	D	D	—	B	C	D	D	—	B	C	D	D	—
“ villi.	A	A	B	C	D	“	B	“	C	D	“	“	C	“	—	“	“	C	“	—	A	B	C	“	—
Jejunum crypt.	0	0	A	B	C	B	C	D	D	—	“	“	D	“	—	“	“	D	“	—	B	C	“	“	D
“ villi.	0	A	B	C	D	0	A	B	C	D	A	“	C	“	—	A	B	C	“	D	—	—	—	C	—
Ileum crypt.	0	“	A	B	C	C	C	D	D	—	B	“	D	“	—	B	C	D	“	—	B	C	D	D	—
“ villi.	A	B	B	C	D	B	“	“	“	—	A	B	C	“	—	A	B	C	“	—	A	—	—	—	—
Colon.	0	A	“	“	“	A	B	C	“	—	B	C	“	“	—	“	C	“	“	—	“	B	C	D	D
Stomach.	A	“	“	“	“	B	C	D	“	—	A	B	“	“	—	“	B	“	“	D	“	“	“	“	“
Liver.	“	B	“	“	“	“	“	C	“	—	“	C	“	“	—	“	“	“	“	—	“	“	“	“	“
Pancreas.	0	A	“	“	“	“	“	“	“	—	B	“	“	“	—	“	“	“	C	D	“	“	A	B	“
Spleen.	A	B	“	“	“	“	“	“	“	—	C	“	D	“	—	B	C	“	D	“	“	“	B	“	“
Kidney.	0	A	“	“	C	A	B	B	C	C	0	A	A	B	C	A	A	B	C	C	B	“	C	C	“

Degree of autolysis indicated as follows (see complete description under Histological method): 0 means zero autolysis. A means slight autolysis. B means well advanced autolysis. C means marked autolysis. D means total autolysis.

tubules and the glomeruli, mainly because of their difference in autolysis; in the liver, the parenchyma cells are contrasted with the bile ducts.

In the normal intestine, the autolytic digestion of the mucosa starts in the tips of the villi and progresses fairly slowly and evenly to the bases of the crypts. Even at the end of 24 hours, the crypt epithelium may be fairly well preserved while the tip epithelium is almost totally autolyzed. In the *radiated animal*, on the other hand, *the crypt epithelium autolyzes at a greater speed than the tip epithelium* and is practically completely autolyzed in 10 to 12 hours or earlier, while even after 12 hours of autolysis the tip epithelium in the same animal may be fairly well preserved (compare Text-figs. 1 and 2).

Discussion of Experiments.

Normal Autolysis.—Three normal dogs are sacrificed under anesthesia. Sections of abdominal viscera are autolyzed at body temperature in 0.9 per cent sodium chloride solution saturated while warm with chloroform as described above. The usual course of digestion is from without inward and the progress is steady and continuous. The digestion in the gastrointestinal sections progresses from the villi to the bases of the crypts. Comparisons are made of the penetration of the autolysis as well as of the progress of cell digestion.

Stomach: The chief and parietal cells of the gastric mucosa are more susceptible to autolysis than are the tip cells. The former are autolyzed rapidly and are totally digested at the end of 10 hours. The tip cells autolyze somewhat more slowly and are completely digested in 24 hours.

Duodenum, *jejunum*, *ileum*, and *colon* are quite similar in the type of their autolysis. The tip epithelium is the first to autolyze. In these control experiments the time may vary from 10 to 24 hours before completion of the digestion. Considerable variation is to be noted in this reaction of the epithelium of the villi. The autolysis of the crypts is usually delayed 4 to 8 hours, after which it proceeds regularly though usually not to completion in 24 hours (Text-figs. 1 and 2).

Spleen pulp is found to autolyze 4 to 8 hours faster than the Malpighian bodies, which, as a rule, are not completely autolyzed by 24 hours. Their centers are fairly well preserved at 12 hours. The pulp at this time is usually pretty well digested.

Pancreas digests pretty well as a unit. The islands of Langerhans autolyze on the average 2 to 4 hours more slowly at first than the parenchyma but later the speed varies considerably so that they may totally digest in 10 hours, or only partly digest in the full 24 hours.

Kidney convoluted tubules are very sensitive to autolysis. Usually they are completely digested in 8 or 10 hours. The remainder of the *kidney parenchyma* consisting of the straight and collecting tubules and the glomeruli is more slowly digested, the full 24 hours usually being required for their complete autolysis.

Liver parenchyma and bile capillaries autolyze as a unit.

Autolysis of Radiated Organs.—Seven normal dogs are exposed to large amounts of radiation, given diffusely over the abdomen, and sacrificed at definite periods after exposure. Autolysis specimens are studied (as above) in regard to the progress of digestion in the specialized cells of the various organs.

Stomach epithelium, though the speed of autolysis may seem to have increased slightly, is digested well within normal limits. This is true for the whole series of dogs which were killed 2, 24, 48, 72, and 96 hours after radiation.

Intestinal epithelium shows a striking change from the controls. The speed of autolysis of the tip epithelium in each case is practically within normal limits. The great change has occurred in the speed of the autolysis of the crypt epithelium. The curves in Text-figs. 1 and 2 illustrate this very well.

The speed of autolysis of the crypt epithelium is rapid and must be due to the immediate effect of radiation upon these cells. The most pronounced increase in the speed of autolysis occurs within the first 2 hours after exposure to radiation. The change produced in these cells, or what is left of them, is permanent up to the time of the death of the animal 4 days later. Even after a lapse of 4 days, these cells autolyze faster than the normal crypt epithelium. These curves correspond remarkably well, indicating that the increase in autolysis is due to a change in the cell protoplasm which persists at least for 4 days after radiation. In all of the animals exposed to radiation the crypt epithelium of the small intestine is completely autolyzed in 12 hours in the duodenum, ileum, and jejunum, while in the experiments with normal animals, the crypt epithelium is rarely autolyzed completely in 24 hours and usually the autolysis is only well advanced by that time.

Colon shows an increase in the speed of autolysis of the crypt epithelium and possibly of the tip epithelium, though the latter is less pronounced. The effect of the radiation seems to decrease or wear off slightly, for the speed of autolysis is somewhat slower and approaches the normal in the 3rd and 4th days after exposure to radiation. This may be connected with the fact that there is also relatively little injury to the colon, anatomically or histologically. The most pronounced increase in the speed of autolysis in the colon epithelium occurs, therefore, within the first 48 hours.

Spleen shows an apparent increase in the speed of autolysis, especially in the first 24 hours after exposure to the x-rays. The 72 hour curve is within normal limits, while both the 48 and the 96 hour curves show an increased speed in autolysis.

Pancreas shows a slight but definite increase in the speed of autolysis, especially during the first 48 hours after exposure. The 96 hour curves seem to indicate also that the speed of autolysis is increased, but the 72 hour curves are irregular as in the spleen series. The islands of Langerhans autolyze as a rule somewhat faster than the pancreatic parenchyma.

Kidney does not show changes in the speed of autolysis after exposure to radiation. The convoluted tubules are totally autolyzed in 8 hours. The other tubules and the glomeruli, while autolyzing more slowly, apparently digest at the normal rate. This would suggest that radiation has little effect upon the kidney.

Liver parenchyma shows a definite increase in the speed of autolysis, with a maximum in the specimens removed 2 hours after the radiation. The 24 and 48 hour experiments show less increase above normal.

DISCUSSION.

Many observers have studied ferment changes *in vitro* in normal organs or tumor tissues exposed to x-rays before the unit period of autolysis. Much of this work has been reviewed by Hall and Whipple and need not be discussed. Our experiments indicate that the spleen, liver, and pancreas taken from a radiated animal will show a more rapid autolysis than similar tissues from control normal animals under similar conditions of autolysis. The kidney shows no such change by the methods used. We may recall that the spleen shows obvious histological injury after exposure to the x-rays, but such changes are lacking in the liver, pancreas, and kidney.

The stomach and colon are much more resistant than the small intestine, but we shall publish experiments to show that large and permanent gastric and colon ulcers may be produced by the x-rays.

The *duration of the injury* done the body cells is of much interest to us. We note that abnormal autolysis of the spleen, liver, and pancreas is most striking within 24 hours after the radiation. After 48 hours, there is a distinct decrease in this reaction as observed in dogs killed 72 and 96 hours following the lethal dose of x-rays. In the small intestine, it is easy to demonstrate the abnormal autolysis in dogs even 96 hours after the x-ray exposure. This indicates in a sense that these cells are suffering from a persistence of the injury exerted by the x-rays, even during the 4th day after the exposure. It is significant that the clinical symptoms always reach a crisis on the 4th day. If this period is survived, the dog usually goes on to recovery.

We may note in passing that the cell necrosis and injury in the intestine as in the skin is associated with a minimal wandering cell reaction. This point deserves further study. We shall record also the fact that ulcers caused by the x-rays are as stubborn in healing in the stomach and intestine as is so well recognized in the skin.

SUMMARY.

Exposure to large doses of x-rays will cause notable increase in the speed of autolysis of the crypt or secretory epithelium of the dog's small intestine. These changes can be demonstrated readily in

material obtained from dogs killed 2, 24, 48, 72, or 96 hours after the initial radiation (Text-figs. 1 and 2).

In the radiated dogs the secretory crypt epithelium of the small intestine autolyzes first and the epithelium of the villi last, while the reverse is true in the normal control small intestine. These abnormalities of autolysis associated with lethal Roentgen ray exposures can be demonstrated for the small intestine over the whole 4 day period subsequent to radiation.

The colon shows little change and the stomach no demonstrable changes in autolysis under like conditions. The kidney likewise is negative.

The spleen, lymph glands, liver, and pancreas show a moderate increase in speed of autolysis in tissues taken from radiated animals within 48 hours of the initial exposure.

What the significance of this disturbance of cell ferments in the intestinal mucosa may be, we cannot pretend to say. At least these observations strengthen one's confidence in the profound functional disturbance of this important intestinal epithelium—a disturbance which we believe is responsible for the clinical abnormalities and fatal intoxication.

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[Reprinted from THE JOURNAL OF EXPERIMENTAL MEDICINE, February 1, 1922,
Vol. xxxv, No. 2, pp. 225-242.]

A STUDY OF RENAL FUNCTION IN ROENTGEN RAY INTOXICATION.

Resistance of Renal Epithelium to Direct Radiation.

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(Received for publication, August 17, 1921.)

In an earlier publication (Hall and Whipple (4)), the noticeable clinical similarity between intestinal obstruction and systemic x-ray intoxication has been pointed out. We have recorded elsewhere (10, 11) an interesting transient disturbance of renal function in the intoxication due to intestinal obstruction and in certain proteose intoxications. This disturbance of renal function is considerable and plays an important part in certain of the clinical reactions (intestinal obstructions). We believe this is the first instance of a well established functional depression of renal secretory function totally unassociated with any abnormalities of renal structure. Because of this peculiar disturbance of renal function in certain proteose intoxications, we took up a study of renal function in systemic x-ray intoxication with the keenest interest. We may say at once that our experiments show a totally different picture, with the renal function uninvolved in the toxic disturbance of general x-ray intoxication. There is a minimal disturbance of renal function by the direct action of very large doses of the hard x-rays but no anatomical evidence of cell injury. We feel confident that the clinician may use the x-rays over the kidney areas with confidence that renal tissue is resistant to the hard Roentgen rays. Our experiments indicate that the kidney epithelium is much more resistant to x-ray injury than is the epithelium of the small intestine.

Inspection of the literature shows that work has been done to determine possible injury of the kidney by the x-rays. However, most of the reports deal with the morphological changes produced or consist of clinical observations made by use of the older, less reliable means of determining the presence of functional disturbances. Warthin (18) noted pathological changes in the kidneys of several leucemia patients who had received x-ray treatment before death and reports finding definite changes in the tubular epithelium following prolonged percutaneous irradiation of the kidneys of white mice and rats. Rosenstern (13), Linser and Helber (9), Schleip and Hildebrand (15), von Jaksch (6), Heymann (5), Přibram and Rotky (12), and others likewise claim to have found evidence of an x ray nephritis. It has been thought by many that the inability of injured kidneys to do their work efficiently must be considered as an essential factor in the constitutional x-ray reaction. While Edsall and Pemberton (3) considered the primary intoxication to be quite independent of any direct effect of the x-rays upon the kidneys, they suggest that the kidneys may become overtaxed by the increased work thrown upon them to such an extent that their failure may secondarily intensify the symptoms of intoxication.

There are many workers, however, who have failed to find any definite clinical or postmortem evidence of an x-ray nephritis. Among those who have reported negative findings may be mentioned Krause and Ziegler (8) and Buschke and Schmidt (2) The latter workers incised the abdominal wall and exposed the kidneys of a number of rats directly to large doses of the x-rays. The animals died a few weeks later, two of them only showing albumin in the urine and pathological changes in the structure of the kidneys at autopsy. Since in these two cases the changes might well have been due to some complication following the operations, these workers concluded that the renal epithelium is very resistant to the x-rays and that consequently there is no such thing as an x-ray nephritis. Warthin, whose work is more recent and freer from complications, admits that the anatomical changes were so slight in his experimental animals that they might be overlooked unless special histological technique is employed. Krause (7) in 300 cases finds no albumin or leucocytes in the urine after radiation. Stephan (16) in a small series of cases claims to cure anuria and produce a diuresis in 24 hours by means of $\frac{1}{8}$ to $\frac{1}{4}$ of an erythema dose over the kidneys. This brief reference to the work previously done on the subject suffices to show the decided lack of agreement among those who have given any attention to it. There is obvious need for a thorough functional study of the problem with employment of the comparatively accurate methods now available for measuring the functional activity of the kidneys under conditions of carefully controlled x-ray administration.

Method.

Normal adult dogs were used exclusively in all of the experiments. The animals were selected after being under observation for a period of several days during which time the functional tests to be used in the subsequent experiments were performed on them. The data recorded in this preliminary examination on each dog serve as a standard with which to compare results obtained in subsequent experiments. At the end of this fore period the animal was exposed to a carefully measured dose of x-rays, following which the renal function was determined daily for a period of a week or more.

The hard Roentgen rays of the Coolidge tube were used exclusively, the softer rays being filtered out with 2 mm. aluminum filters. In a very few cases only did we have leakage with slight tendency to pigmentation and denudation of the skin. All exposures were percutaneous and the distance from the target (anticathode) to the surface of the animal's body measured in all cases 25 cm. The ammeter was made to read at approximately 8 milliamperes in most of the experiments, although currents as high as 10 milliamperes and as low as 5 milliamperes were employed in a few instances. Our work confirms the observation of Hall and Whipple (4) that in dogs the toxicity of the hard rays increases with the increase in spark-gap. Our experience has shown that a 9 inch spark-gap is a convenient one for producing the desired experimental intoxication and that this required an E. M. F. of about 90 kilovolts. With a few exceptions, this voltage was employed.

In a part of the experiments moderate sized sublethal doses of x-rays were given at intervals over a considerable period of time, a short rest on a mixed diet being given between exposures. In the remainder, huge and frequently fatal doses were given singly or in succession. It was considered that between these extremes no impairment of function, whether prompt or delayed in its appearance, could escape detection. In order to understand the nature or the mechanism of any disturbance which might be observed, different regions of the body were exposed separately in the various experiments.

For a period of 1 to 3 days preceding and 5 to 6 days subsequent to the radiation, the dogs were given only water. During resting intervals, the dogs received the usual kennel mixed diet of table scraps, cooked meat, bread, rice, bones, and so forth.

The methods chosen for the measurement of the renal function were those employed by us in studies on the renal function in intestinal obstruction (10); namely, the phenolsulfonephthalein elimination test (14) and that of determining the capacity of the kidneys to excrete urea. The former method was carried out according to the procedure outlined by its authors, the Duboscq colorimeter being used for the determination of the dye. For the latter we made a daily determination of the blood urea in all experiments and determined in many cases the ratio

$$\frac{\text{Urea in 1 hr.'s urine}}{\text{Urea per 100 cc. of blood}}$$

after the intravenous injection of comparatively large amounts of urea in saline solution. A decrease in this ratio indicates a definite impairment of kidney function as shown by Addis and Watanabe (1). The tests were made after the exposure to the x-rays exactly as in the fore period except that in cases of extreme intoxication with resulting loss of fluid from the body, more fluid was given intravenously in order that the loss might be made up as far as possible. The urea of both the blood and the urine was determined by the Van Slyke-Cullen modification of the Marshall urease method (17). Morphine sulfate ($\frac{1}{8}$ to $\frac{1}{4}$ gr. doses) was administered subcutaneously 1 hour before the animal was placed upon the x-ray table to insure quiet during the period of exposure.

EXPERIMENTAL DATA.

The experimental data assembled below consist of three types of experiments. The first includes a group of observations on several different animals in which *sublethal doses of x-rays* were given at intervals over relatively long periods of time. In these experiments, the thorax and the abdomen, including the kidneys, were alternately exposed. The purpose of this series was to ascertain whether or not repeated x-ray treatment over a long period of time is capable of producing any gradual changes in the renal function. Many have feared the possibility of cumulative action resulting in permanent damage to the kidneys.

Dog 19–85 (*Tables I and II*). *Autopsy Record.*—Dog sacrificed under ether anesthesia 4 days after the last radiation, Apr. 5. The kidneys in gross are quite normal. Bladder normal. Histological sections show normal stroma, vessels, and glomeruli. There are no scars. Convoluted tubules are normal

TABLE I.

Large Sublethal Doses of X-Rays Given over Abdomen and Thorax. Renal Function Unimpaired.

Dog 19–85. Adult female spitz.

Date.	Weight.	Blood urea per 100 cc.	Phthalein elimination in 2 hrs.	Remarks.
	lbs.	*mg.*	*per cent*	
Nov. 30	24.9	23	75	Normal.
Nov. 30	190 milliampere minutes, x-rays over abdomen and kidneys; 90 kilovolts; 10 milliamperes.			
Dec. 1	24.7	22	71	Normal.
" 3	24.2	36	74	"
" 5	23.7	34	54	Slight diarrhea.
" 6	23.2	36	55	No diarrhea. Normal.
" 7	22.7	40	63	Normal.
Dec. 16	220 milliampere minutes, x-rays over thorax and neck; 90 kilovolts; 10 milliamperes			
Dec. 17	24.3	32	62	Normal.
" 18	23.9	30	69	—
" 19	23.1	42	57	—
" 20	23.0	31	52	—
" 21	22.9	30	67	Normal.
Dec. 31	350 milliampere minutes, x-rays over abdomen and back; 90 kilovolts; 8 milliamperes.			
Jan. 1	22.6	—	67	Normal.
" 2	22.0	22	67	—
" 3	21.8	20	71	—
" 4	21.2	23	63	Normal.
" 5	20.8	30	67	—
" 6	20.4	28	63	—
Feb. 4	300 milliampere minutes, x-rays over abdomen and back; 95 kilovolts; 8 milliamperes.			
Feb. 8	23.0	34	63	Normal.
" 9	23.0	30	63	—
" 10	22.6	28	66	—
" 11	22.4	24	64	—
" 12	22.2	25	63	—

Mixed diet in intervals between experiments; no food for 5 days following exposures.

It will be seen from Tables I to IV that the experiments of this group are a unit in showing that the excretory function of the kidneys, as measured by their ability to eliminate a foreign substance such as phenolsulfonephthalein or the normal urinary constituent, urea, is not disturbed by this procedure. It is evident that with the doses given there is no sign of any cumulative effect being produced, whether the thorax or abdomen or both be exposed.

TABLE II.

Large Sublethal Doses of X-Rays Given over Abdomen and Thorax. Renal Function Unimpaired.

Dog 19–85. Adult female spitz.

Date.	Hr.	Urea injected.	Urea excreted per hr.	Blood urea per 100 cc.	Ratio.	Phthalein elimination in 2 hrs.	Remarks.
		gm.	*gm.*	*mg.*		*per cent*	
Dec. 21	9.00–10.00	—	—	30	—	—	Normal.
	10.00–12.00	20	3.59	262	13.7	67	—
Mar. 4	350 milliampere minutes, x-rays over thorax; 95 kilovolts; 5 milliamperes.						
Mar. 7	9.00–11.00	—	—	28	—	63	Normal.
" 8	—	—	—	27	—	64	—
" 9	10.00–12.00	20	3.78	270	14.0	67	Normal.
Apr. 1	350 milliampere minutes, x-rays over abdomen; 105 kilovolts; 5 milliamperes.						
Apr. 4	12.00–2.00	—	—	39	—	65	Moderate intoxication.
" 5	3.00–5.00	20	3.42	278	12.3	—	Same condition.

The second type of experiment is represented by the observations made on Dogs 19–127 and 19–140 (Tables V to VIII) in which a lethal dose of the x-rays was given over the abdomen with the kidneys and the remainder of the body protected from the direct rays by the leaden sheets. This type of experiment was designed to demonstrate whether or not a poisonous substance capable of injuring the kidneys is produced in the irradiated gastrointestinal tract and carried by the blood stream to the kidneys. Reference to the tables shows that the function of the kidneys remains entirely normal after the exposure,

TABLE III.

Repeated Sublethal Doses of X-Rays over Thorax and Abdomen. Renal Function Unchanged.

Dog 19–78. Adult female Airedale. (Autopsy, see Table XV.)

Date.	Weight.	Blood urea per 100 cc.	Phthalein elimination in 2 hrs.	Remarks.
	lbs.	*mg.*	*per cent*	
Nov. 23	28.5	30	68	Normal.
Nov. 25	190 milliampere minutes, x-rays over thorax and neck; 85 kilovolts; 5 milliamperes.			
Nov. 26	28.1	18	63	Normal.
“ 27	27.5	22	57	—
“ 28	27.4	20	60	—
“ 29	27.1	—	58	—
“ 30	26.5	33	58	—
Dec. 1	26.4	28	60	—
“ 2	26.4	23	68	No food since exposure.
“ 5	26.0	20	60	Mixed diet.
“ 10	27.5	—	62	—
Dec. 10	190 milliampere minutes, x-rays over abdomen and back; 90 kilovolts; 5 milliamperes.			
Dec. 11	27.5	40	59	Normal.
“ 12	27.0	40	59	—
“ 13	26.2	40	54	—
“ 14	26.0	41	59	—
“ 15	25.7	36	54	—
“ 16	24.9	22	59	—
Dec. 27	240 milliampere minutes, x-rays over thorax; 90 kilovolts; 5 milliamperes.			
Dec. 28	26.6	36	64	Normal.
“ 29	26.5	47	64	—
“ 30	26.3	50	62	—
“ 31	25.5	43	63	—
Jan. 1	25.0	33	59	—

Dog not fed for day or two before exposure and for 5 days after each radiation.

although the dose of x-rays produced profound intoxication and death. The evidence against any indirect action of the x-rays upon the kidney function or upon the structure of the kidney is very clear-cut. The blood urea showed a definite increase in both cases, but this was due to accelerated tissue destruction rather than to retention, as is shown by the fact that the urea ratio (Tables VI and VIII) remained normal even when additional urea was injected intravenously. The results indicate the absence of any hypothetical nephrotoxic substance in the blood during the period of intoxication, and thus point out a

TABLE IV.

Repeated Increasing Doses of X-Rays over Thorax and Abdomen. Renal Function Undisturbed.

Dog 19–50. Young adult female. (Autopsy, see Table XVI.)

Date.	Weight.	Blood urea per 100 cc.	Phthalein elimination in 2 hrs.	Remarks.
	lbs.	*mg.*	*per cent*	
Dec. 5	30.40	28	74	—
Dec. 5	210 milliampere minutes, x-rays over thorax; 90 kilovolts; 10 milliamperes.			
Dec. 6	29.50	22	72	Normal.
“ 7	29.30	31	66	—
“ 8	28.20	31	63	—
“ 9	27.00	34	55	—
“ 10	26.40	34	63	—
“ 12	26.50	—	—	—
“ 14	26.50	28	70	—
“ 15	27.10	33	—	—
Dec. 16	210 milliampere minutes, x-rays over abdomen and back; 90 kilovolts; 10 milliamperes.			
Dec. 17	27.15	37	56	Normal.
“ 18	27.00	31	68	—
“ 19	26.70	34	65	—
“ 20	25.40	30	63	—
Dec. 31	350 milliampere minutes, x-rays over thorax; 90 kilovolts; 7.5 milliamperes.			
Jan. 1	26.70	—	72	Normal.
“ 2	26.50	28	67	—
“ 3	26.00	26	61	—
“ 4	25.50	22	61	—
“ 5	25.40	24	63	—
“ 6	25.30	20	66	—
“ 7	25.00	21	63	—

TABLE V.

Lethal Dose of X-Rays over Abdomen with Kidneys Shielded. Renal Function Unchanged.

Dog 19–127. Adult male mongrel.

Date.	Weight.	Blood urea per 100 cc.	Phthalein elimination in 2 hrs.	Remarks.
	lbs.	*mg.*	*per cent*	
June 9	28.4	32	74	
" 10	28.3	30	72	Normal.
June 10	504 milliampere minutes, x-rays over abdomen, kidneys completely shielded; 90 kilovolts; 8 milliamperes.			
June 11	27.8	27	76	Normal.
" 12	26.3	35	67	Quiet.
" 13	26.1	44	68	Dull and weak.
" 14	25.5	46	66	Intoxication increasing.
" 15	—	53	59	Grave intoxication and collapse. Death in night.

TABLE VI.

Lethal Dose of X-Rays over Abdomen with Kidneys Shielded. Renal Function Unchanged.

Dog 19–127. Adult male mongrel.

Date.	Hr.	Urea injected.	Urea excreted per hr.	Blood urea per 100 cc.	Ratio.	Phthalein elimination in 2 hrs.	Remarks.
		gm.	*gm.*	*mg.*		*per cent*	
June 9	8.50– 9.50	—	0.160	32	5.0	—	—
	10.00–11.00	20	2.382	240	9.9	74	Normal.
	11.00–12.00	—	1.856	201	9.2	—	—
June 10	504 milliampere minutes, x-rays over abdomen, kidneys shielded; 90 kilovolts; 8 milliamperes.						
June 14	8.45– 9.45	—	0.144	46	3.1	—	—
	10.00–11.00	20	2.215	237	9.3	66	Severe intoxication.
	11.00–12.00	—	1.693	192	8.8	—	—

TABLE VII.

Lethal Dose of X-Rays over Abdomen with Kidneys Shielded. Renal Function Unchanged.

Dog 19–140. Young adult female collie.

Date.	Weight.	Blood urea per 100 cc.	Phthalein elimination in 2 hrs.	Remarks.
	lbs.	*mg.*	*per cent*	
July 7	21.7	34	70	—
" 8	20.5	32	72	Normal.
July 8	450 milliampere minutes, x-rays over abdomen, kidneys shielded; 90 kilovolts; 8 milliamperes.			
July 9	19.7	30	70	Normal.
" 10	19.3	34	63	Dull and weak.
" 11	19.0	43	63	Severe intoxication; bloody diarrhea; death in night.

TABLE VIII.

Lethal Dose of X-Rays over Abdomen with Kidneys Shielded. Renal Function Unchanged.

Dog 19–140. Young adult female collie.

Date.	Hr.	Urea injected.	Urea excreted per hr.	Blood urea per 100 cc.	Ratio.	Phthalein elimination in 2 hrs.	Remarks.
		gm.	*gm.*	*mg.*		*per cent*	
July 8	7.55– 8.55	—	0.160	32	5.0	—	—
	9.00–10.00	20	3.028	244	12.4	72	Normal.
	10.00–11.00	—	2.353	219	10.7	—	—
July 8	450 milliampere minutes, x-rays over abdomen, kidneys shielded; 90 kilovolts; 8 milliamperes.						
July 11	8.30– 9.30	—	0.206	43	4.8	—	—
	10.00–11.00	20	2.744	240	11.4	68	Typical x-ray intoxication.
	11.00–12.00	—	2.366	213	11.1	—	—

difference between this type of intoxication and that of acute intestinal obstruction in which a transient impairment of kidney function is constantly found.

TABLE IX.

Large Doses of X-Rays Directly over Kidneys with Abdomen Shielded. Kidney Function Slightly Impaired.

Dog 19–117. Adult female setter.

Date.	Weight.	Blood urea per 100 cc.	Phthalein elimination in 2 hrs.	Remarks.
	lbs.	*mg.*	*per cent*	
July 28	38.8	32	67	Normal.
July 28	456 milliampere minutes, x-rays directly over kidneys, other parts shielded; 90 kilovolts; 8 milliamperes.			
July 29	38.8	30	68	Normal.
" 30	38.4	29	66	—
" 31	38.0	27	66	Bread and milk diet.
Aug. 1	37.6	28	63	Dog a little sick.
" 2	37.4	24	63	—
" 3	37.8	—	—	—
" 4	37.4	26	48	—
" 5	37.0	33	32	—
" 6	36.4	—	39	—
" 10	—	33	49	Normal.
" 11	36.5	33	56	—
Aug. 12	512 milliampere minutes, x-rays over both kidneys with other parts shielded; 90 kilovolts; 8 milliamperes.			
Aug. 13	36.3	26	59	—
" 14	36.2	30	—	Dog fed; good condition.
" 15	36.0	30	55	—
" 16	36.1	—	56	—
" 17	36.2	32	48	—
" 18	36.0	38	45	Small amount of vomitus.
" 19	36.0	—	42	Moderate diarrhea.
" 20	35.2	32	58	Clinical improvement.
" 21	35.0	—	—	Normal.

Dog 19–127 (*Tables V and VI*). *Autopsy Record.*—Dog died in night of 4th day after exposure to a lethal dose of x-rays given over abdomen with shielding of the kidneys. Autopsy next morning. Kidneys and bladder normal in gross. Histological sections show some postmortem changes in the epithelium of the

convoluted tubules. The stroma and glomeruli are normal. There are no scars. The vessels are filled with blood.

The third type of experiment is represented by the series of observations on Dogs 19-117, 19-129, and 18-115 (Tables IX to XIV).

TABLE X.

Large Dose of X-Rays Directly over Kidneys with Abdomen Shielded. Kidney Function Slightly Impaired.

Dog 19-117. Adult female setter.

Date.	Hr.	Urea injected.	Urea excreted per hr.	Blood urea per 100 cc.	Ratio.	Phthalein elimination in 2 hrs.	Remarks.
		gm.	*gm.*	*mg.*		*per cent*	
July 14	12.50–1.50	—	0.170	30	5.6	—	—
	2.00–3.00	20	1.799	158	11.4	74	Normal.
	3.00–4.00	—	1.323	142	9.3	—	—
July 15	456 milliampere minutes, x-rays over thorax and neck; 90 kilovolts; 8 milliamperes.						
July 18	8.45– 9.45	—	0.160	27	5.9	—	—
	10.00–11.00	20	1.826	159	11.5	67	Normal.
	11.00–12.00	—	1.357	144	9.4	—	—
July 28	456 milliampere minutes, x-rays over kidneys, other parts shielded; 90 kilovolts; 8 milliamperes.						
Aug. 11	2.50–3.50	—	0.160	33	4.8	—	—
	4.00–5.00	20	1.505	173	8.7	56	Not clinically sick.
	5.00–6.00	—	1.182	163	7.3	—	—
Aug. 12	512 milliampere minutes, x-rays over kidneys, other parts shielded; 90 kilovolts; 8 milliamperes.						
Aug. 18	1.45–2.45	—	0.174	38	4.5	—	—
	3.00–4.00	20	1.342	179	7.4	45	Dull. Small amount vomitus.
	4.00–5.00	—	1.316	167	7.8	—	—

In these the kidneys were exposed to huge doses of the x-rays directly while the remainder of the abdomen and the thorax were completely shielded. Large doses were repeatedly given in the case of the first animal while large single doses only were given in the other cases.

TABLE XI.

Large Dose of X-Rays over Kidneys with Abdomen Shielded. Kidney Function Slightly Impaired.

Dog 19–129. Adult male mongrel.

Date.	Weight.	Blood urea per 100 cc.	Phthalein elimination in 2 hrs.	Remarks.
	lbs.	*mg.*	*per cent*	
Aug. 6	67.9	29	66	Normal.
" 7	67.4	28	67	—
Aug. 8	512 milliampere minutes, x-rays over thorax; 90 kilovolts; 8 milliamperes.			
Aug. 10	66.7	26	66	Normal.
" 12	65.0	29	63	—
" 14	64.8	25	64	Mixed diet.
Aug. 19	512 milliampere minutes, x-rays directly over kidneys, with other parts shielded; 90 kilovolts; 8 milliamperes.			
Aug. 19	63.0	29	67	Normal.
" 22	61.7	38	53	Slightly dull.
" 24	59.7	46	44	Little vomitus.
" 26	59.3	44	54	Slightly dull.
" 28	59.0	32	58	Normal.

TABLE XII.

Large Dose of X-Rays over Kidneys with Abdomen Shielded. Kidney Function Slightly Impaired.

Dog 19–129. Adult male mongrel.

Date.	Hr.	Urea injected.	Urea excreted per hr.	Blood urea per 100 cc.	Ratio.	Phthalein elimination in 2 hrs.	Remarks.
		gm.	*gm.*	*mg.*		*per cent*	
Aug. 19	8.40– 9.40	—	0.142	29	4.9	—	—
	10.00–11.00	20	1.368	143	9.5	66	Control.
	11.00–12.00	—	1.083	129	8.4	—	—
Aug. 19	512 milliampere minutes, x-rays over kidneys with abdomen shielded; 90 kilovolts; 8 milliamperes.						
Aug. 26	—	—	—	47	—	—	—
	1.00–2.00	20	1.124	161	6.9	47	Slightly dull.
	2.00–3.00	—	0.933	150	6.2	—	—

Dog 19–129 showed no signs of intoxication, whereas No. 19–117 showed a slight reaction and No. 18–115 was markedly intoxicated. It is probable that in the case of No. 18–115 a small portion of the intestine was included in the area of exposure. It is to be noted that the doses given over the kidneys in this group of experiments were at least 25 per cent greater than the lethal dose over the abdomen.

TABLE XIII.

Large Dose of X-Rays over Kidneys with Abdomen Shielded.
Kidney Function Slightly Impaired.

Dog 18–115. Adult female bulldog.

Date.	Weight.	Blood urea per 100 cc.	Phthalein elimination in 2 hrs.	Remarks.
	lbs.	*mg.*	*per cent*	
Aug. 23	21.5	30	68	Normal.
" 24	20.2	27	66	"
Aug. 27	512 milliampere minutes, x-rays over kidneys with remainder of body shielded; 90 kilovolts; 8 milliamperes.			
Aug. 28	19.4	32	62	Normal.
" 29	19.2	31	54	—
" 30	19.0	33	58	Normal.
" 31	—	—	58	Slightly dull.
Sept. 1	18.3	52	40	Quiet; vomitus and tarry stools
" 2	17.9	43	—	Tarry stools and vomitus
Sept. 3	300 milliampere minutes, x-rays over abdomen alone; 90 kilovolts: 8 milliamperes.			
Sept. 4	17.2	36	53	Vomits food; tarry stools.
" 5	16.7	44	46	Weak and dull.
" 8	—	—	—	Found dead in cage

Dog 19–117 (Tables IX and X). Autopsy Record.—Dog sacrificed under ether Aug. 24 and autopsy done at once. Kidneys and bladder normal in gross. Histological sections show normal glomeruli, vessels, and stroma. There are no scars. Convoluted tubules show normal epithelium. There are a few pale hyaline casts in the collecting tubules of the pyramids.

Dog 19–129 (Tables XI and XII). Autopsy Record.—Dog sacrificed with ether anesthesia Aug. 29 and autopsy done at once. Kidneys show definite chronic nephritis with a large cyst in the left kidney. There is a chronic granular cystitis. Histological sections show a marked cellular pyelitis in both kidneys. There are a few cellular scars in the cortex and some large fibrous scars (arteriosclerotic) involving a considerable area of the cortex and showing tubular and glomerular destruction. There is no evidence of acute nephritis. Outside the scar areas, the glomeruli and tubules appear to be quite normal. This picture is often found in the kidneys of old dogs and cannot in any way be related to the x-ray exposures. This abnormal kidney seems to be no more susceptible to radiation than the normal kidney.

Dog 18–115 (*Tables XIII and XIV*). *Autopsy Record.*—Dog found dead in cage Sept. 8 and autopsy done at once. Kidneys are normal in gross. Histological sections show normal glomeruli and vessels. There are a few cellular scars in cortex. The convoluted tubules show a slight postmortem change, but otherwise the tubules are normal.

TABLE XIV.

Large Dose of X-Rays over Kidneys with Abdomen Shielded. Kidney Function Slightly Impaired.

Dog 18–115. Adult female bulldog.

Date.	Hr.	Urea injected.	Urea excreted per hr.	Blood urea per 100 cc.	Ratio.	Phthalein elimination in 2 hrs.	Remarks.
		gm.	*gm.*	*mg.*		*per cent*	
Aug. 24	2.30–3.30	—	0.153	27	5.6	—	—
	4.00–5.00	20	2.562	197	13.1	66	Control.
	5.00–6.00	—	1.998	181	11.0	—	—
Aug. 27	512 milliampere minutes, x-rays over kidneys with remainder of body shielded; 90 kilovolts; 8 milliamperes.						
Sept. 1	1.30–2.30	—	0.203	52	3.9	—	—
	3.00–4.00	20	2.014	217	9.2	40	Dog is weak. Tarry stools and vomitus.
	4.00–5.00	—	1.683	179	9.4	—	—

Tables IX to XIV inclusive, in which the data of these experiments are recorded, show that the direct exposure of the kidneys to massive doses of the x-rays gives rise to a moderate but definite depression of the kidney function. In Tables XV and XVI are shown the results of two experiments differing slightly from the foregoing in that the kidneys and the intestines were together exposed to large doses of x-rays, the thorax only being protected. A definite decrease in the 2 hour output of phenolsulfonephthalein and a corresponding drop in the urea ratio indicate a moderate impairment of renal function.

TABLE XV.

Lethal Dose of X-Rays over Kidneys and Abdomen.
Renal Function Moderately Impaired.

Dog 19–78. Adult female Airedale.

Date.	Hr.	Urea injected.	Urea excreted per hr.	Blood urea per 100 cc.	Ratio.	Phthalein elimination in 2 hrs.	Remarks.
		gm.	*gm.*	*mg.*		*per cent*	
Dec. 7	1st	—	—	24	—	—	—
	2nd	20	1.826	152	12.0	69	Control.
	3rd	—	1.594	131	12.1	—	—
Mar. 18	350 milliampere minutes, x-rays over abdomen and kidneys; 95 kilovolts; 8 milliamperes.						
Mar. 20	—	—	—	32	—	55	Slightly dull.
" 21	—	—	—	45	—	40	Intoxication progressing.
Mar. 22	1st	—	—	69	—	—	—
	2nd	20	1.861	258	7.2	38	Marked intoxication; bloody diarrhea.
	3rd	—	1.443	239	6.0	—	—

Dog killed under ether at end of observation.

TABLE XVI.

Lethal Dose of X-Rays over Kidneys and Abdomen.
Renal Function Slightly Impaired.

Dog 19–50. Young adult female.

Date.	Hr.	Urea injected.	Urea excreted per hr.	Blood urea per 100 cc.	Ratio.	Phthalein elimination in 2 hrs.	Remarks.
		gm.	*gm.*	*mg.*		*per cent*	
Feb. 10	1st	—	0.240	29	4.8	—	—
	2nd	20	1.983	180	11.0	66	Control.
	3rd	—	1.529	156	9.8	—	—
Feb. 11	340 milliampere minutes, over abdomen and kidneys; 95 kilovolts; 8 milliamperes.						
Feb. 13	—	—	—	42	—	56	Dull; vomitus and diarrhea.
Feb. 14	1st	—	0.328	78	4.2	—	—
	2nd	20	2.146	280	7.66	40	Frequent diarrhea and vomiting.
	3rd	—	1.543	249	6.19	—	—

From the results of the experiments outlined above we assume that the direct action of the x-rays upon the kidneys is responsible for this depression of activity.

Dog 19-78 (*Tables III and XV*). *Autopsy Record.*—Dog killed under ether at end of experiment given in Table XV. Autopsy done at once but only the renal findings given here. Kidneys are normal in gross. Histological sections show normal glomeruli, vessels, and stroma. There are no scars. Convoluted tubules are normal. The epithelium of the loops of Henle shows a frothy looking protoplasm but this picture is seen in control dogs.

Dog 19-50 (*Tables IV and XVI*). *Autopsy Record.*—Dog killed under ether anesthesia at end of last experiment (Table XVI) and autopsy at once. Only the renal findings are given here. Kidneys and bladder normal in gross. Histological sections show normal glomeruli and vessels. There are a few small cellular scars in the cortex. Tubules are normal.

Reference to the autopsy findings in the various dogs used in the experiments shows that no constant anatomical change is seen in the kidneys which would serve to distinguish them from a series of normal control kidneys chosen at random. We did not use the refined histological technique recommended by Warthin, but we are inclined to believe that much of the histological evidence submitted in favor of x-ray injury of the kidney is due to kidney abnormalities present *before the x-ray exposures*. Such abnormalities would be revealed by a careful study of suitable controls.

SUMMARY.

Our experiments give no support to the current belief that an x-ray nephritis may be produced by direct or indirect action of the hard Roentgen rays.

Moderate doses of x-rays given *repeatedly* over considerable periods of time have no demonstrable influence on renal function or renal structure.

With x-ray exposures of the abdomen and shielding of the kidneys fatal intoxication may be produced without the slightest disturbance of kidney function as measured by the ability of the kidney to eliminate phenolphthalein and urea.

Large doses of the x-rays given directly over the kidney may cause a slight but distinct lowering of renal function which lasts for a period of a few days. We have been unable to recognize any corresponding histological change.

We feel that the usual therapeutic doses of the x-rays can be given over the kidneys without apprehension. The renal tissue is much more resistant to x-rays than is the epithelium of the small intestine.

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Reprinted from THE AMERICAN JOURNAL OF PHYSIOLOGY
Vol. 61, No. 1, June, 1922

I. DETERMINATION OF PLASMA AND HEMOGLOBIN VOLUMES AFTER UNIT HEMORRHAGES UNDER CONTROLLED EXPERIMENTAL CONDITIONS

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Received for publication March 11, 1922

Much experimental work has been done to study the effect of hemorrhage upon blood volume: observations on experimental animals and human patients after hemorrhage, with or without shock. Much of this work has been adequately reviewed by Bock (2) and we refer to his paper for the bibliography and a critical review of papers.

Our work here reported is somewhat different from that published by other investigators in that we made *simultaneous plasma volume and hemoglobin volume determinations* using methods previously described and controlled as to accuracy under the conditions of these experiments. The dye and hemoglobin method is described by Lee and Whipple (4). The carbon monoxide method is described by Arnold, Carrier, Smith and Whipple (1). The various limitations of these methods are reviewed with experimental control by Smith, Arnold and Whipple (5). This last paper pointed out the important fact that in the dog we record a plasma volume of approximately 5 per cent and a hemoglobin or red cell volume of approximately 4 per cent which indicates a total blood volume of 9 per cent. With the red cell hematocrit as 50 per cent we fall into error and usually calculate the total blood volume from the plasma figures as 10 per cent or from the carbon monoxide figures as 8 per cent making the error of assuming the ratio of cells to plasma remains constant in all parts of the blood stream as 50 per cent or the jugular red cell hematocrit value. We gave evidence that the axial stream in the arterioles and the flow through the capillaries can explain the difference and show where the extra 1 per cent of plasma is to be found. For a review and discussion of all this data we must refer the reader to the original paper (5).

It is becoming increasingly evident that the blood volume figures may be different in various animals of different species. We are familiar with the observations that the red and white cell counts may be very different in normal human beings, normal dogs and normal rabbits. Why should we assume that the circulating blood volume of these different animals should be a constant, whether calculated on the basis of body weight or body surface? We believe it wise to make no assumptions as to normal blood volume figures until accurate determinations have been made in man, dogs, rabbits, monkeys, etc. For accurate work it is obviously desirable to have direct determination figures both for the plasma volume and the hemoglobin volume.

Experimental observations. Dogs were used in all these experiments. These dogs were all healthy active adults. They had been long under observation in the laboratory and used for experiments of similar nature. They were familiar with laboratory and blood volume routine and were quite undisturbed by these procedures. They were receiving a liberal diet of hospital food scraps including meat, bones, potato, bread and milk, which has been shown to be a diet favorable for a rapid regeneration of red cells and hemoglobin after anemia (6). The dogs were fasted for 24 hours preceding the hemorrhage experiments to insure a waterclear plasma but water was in the cages at all times. These procedures caused the dogs no discomfort. Hemorrhage was produced by means of a hypodermic needle introduced into the jugular vein, the blood being aspirated into a flask containing isotonic oxalate solution. This hemorrhage blood was then measured and its hematocrit value determined.

The *tables* are of uniform type and give the necessary data. The dogs, unless note is made to the contrary, were not disturbed by a 25 per cent hemorrhage. *Figures* given in *bold type* indicate the *measured values;* for example, the CO method measures the red cell volume but the plasma volume by this method is *calculated.* In the same way the plasma volume only is *measured* by the dye method. The *true blood volume* is the sum of these two figures for plasma and red cell volume and is given in bold type. The *true red cell hematocrit* is calculated on the basis of the true blood volume and the red cell volume figures. This figure is a theoretical value which would be given if all the blood cells and blood plasma could be removed from a dog, thoroughly mixed, and a sample centrifugalized as usual.

We must not forget that about 8 to 10 per cent of body hemoglobin is *immobilized hemoglobin,* not truly circulating but taking part in diluting the inhaled CO. This immobilized hemoglobin is contained in the muscles (myohematin) and bone marrow. This figure for immobilized hemoglobin is not allowed for in our tables as it probably

TABLE 1

Simultaneous blood volume determinations (plasma and red cells) after 2 bleedings of 25 and 20 per cent of true blood volume

Dog 21–56. White bull dog, female, adult

DATE	METHOD	TOTAL BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	EXPECTED VOLUME AFTER HEMORRHAGE		JUGULAR R. B. C. HEMATOCRIT	VOLUME PER KILO			TRUE BLOOD VOLUME	TRUE R. B. C. HEMATOCRIT	WEIGHT
					Plasma	Cell		Total blood volume	Plasma volume	R. B. C. volume			
1921		*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*	*kilos*
1/27	Hgb.	1461	**658**	803			55.0	116.1	52.2	63.9		46.5	12.6
	CO	1008	437	**571**			56.6	80.0	34.7	45.3	**1229**		
1/31	Hgb.	1335	**633**	702			52.6	106.0	50.2	55.8		46.0	12.6
	CO	979	439	**540**			55.2	77.7	34.8	42.9	**1173**		
2/1	5:00 p.m. Bled total 300 cc. Cells 160 cc. Plasma 140 cc. = 25 per cent of true blood volume												
2/2	Hgb.	1290	**706**	584	493		45.2	102.2	56.1	46.1		34.3	12.6
2:00p.m.	CO	839	470	**369**		360	44.0	66.6	37.3	29.3	**1075**		
2/2	4:15 p.m. Bled total 215 cc. Cells 86 cc. Plasma 129 cc. = 20 per cent of true blood volume												
2/3	Hgb.	1275	**899**	376	577		29.5	101.1	71.3	29.8		25.3	12.6
2:00p.m.	CO	960	655	**305**		269	31.8	76.2	52.0	24.2	**1204**		
2/4	Hgb.	1265	**896**	369			29.3	100.2	71.1	29.1		25.4	12.6
2:30p.m.	CO	1013	707	**306**			30.2	80.4	56.1	24.3	**1202**		
2/7	Hgb.	1146	**786**	360			31.3	91.0	62.4	28.6		31.0	12.6
2:30p.m.	CO	1033	681	**352**			34.1	82.0	54.0	28.0	**1138**		
2/11	Hgb.	1339	**864**	475			35.5	106.2	68.5	37.7		30.8	12.6
3:00p.m.	CO	1023	639	**384**			37.5	81.2	50.8	30.4	**1248**		

does not change greatly during the periods of these experiments. Does the immobilized hemoglobin all become saturated with CO to the same extent as does the hemoglobin of the rapidly circulating red cells,—for example the hemoglobin in the relatively inaccessible bone marrow? We hope to report experiments which will give data as to this immobilized hemoglobin and the fluctuation of muscle hemoglobin.

Unit hemorrhage followed in 18 to 24 hours by blood volume determinations. A considerable number of experiments was performed but only four are given in summary table 3. Some of these experiments lacked a detail here or there and in some the plasma volume alone or the hemoglobin volume alone were measured. All these data are in harmony and the examples cited are truly representative.

TABLE 2

Simultaneous blood volume determinations (plasma and red cells) after 2 bleedings of 25 per cent and 17 per cent of true blood volume

Dog 21–64. Bull terrier, adult, female

DATE	METHOD	TOTAL BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	EXPECTED VOLUME AFTER HEMORRHAGE		JUGULAR R. B. C. HEMATOCRIT	VOLUME PER KILO			TRUE BLOOD VOLUME	TRUE R. B. C. HEMATOCRIT	WEIGHT
					Plasma	Cell		Total blood volume	Plasma volume	R. B. C. volume			
1921		*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*	*kilos*
2/1 3:30 p.m.	Hgb.	1255	**653**	602			48.0	129.3	67.3	62.0		34.6	9.7
	CO	752	406	**346**			46.0	77.5	41.9	35.6	**999**		
2/1	4:30 p.m. Bled 250 cc. Cells 112 cc. Plasma 138 cc. = 25 per cent of true blood volume												
2/2 12:00 p.m.	Hgb.	1023	**692**	331	515		32.4	106.5	72.0	34.5		24.9	9.6
	CO	696	468	**228**		220	32.7	72.5	48.8	23.7	**920**		
2/2	3:00 p.m. Bled 162 cc. Cells 53 cc. Plasma 109 cc. = 17 per cent of true blood volume												
2/3 3:00 p.m.	Hgb.	1017	**752**	265	583		26.1	101.7	75.2	26.5		21.6	10.0
	CO	776	568	**208**		163	26.8	77.6	56.8	20.8	**960**		

Table 1 is a good example of a characteristic experiment. The first hemorrhage was preceded by two control determinations. The determinations after the first hemorrhage were made 21 hours following the bleeding. The second bleeding was done just after these blood determinations on the same day and the blood volume determinations again made 22 hours after the second hemorrhage.

The 20-hour interval between each bleeding and blood volume measurement allowed complete compensation to take place and we note very little change in *total volume*. This means, of course, an obvious *increase* in plasma volume after each hemorrhage, more marked after the second hemorrhage. There is a slight increase in red cell volume after the second bleeding—compare red cell volume by the CO method, 305 cc. with the expected red cell volume 269 cc.

SUMMARY TABLE 3

Simple hemorrhage experiments with simultaneous blood plasma and red cell volume determinations. Compare measured and expected cell and plasma volumes

DOG NUMBER	WEIGHT	TIME	JUGULAR R. B. C. HEMATOCRIT	TRUE VOLUME COMBINED METHOD	PLASMA VOLUME HEMOGLOBIN METHOD	R. B. C. VOLUME CO METHOD	EXPECTED VOLUME Plasma	EXPECTED VOLUME Cell	AMOUNT OF BLEEDING IN PER CENT OF BLOOD VOLUME
	kgm.		*per cent*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*
21–6	13.7	Before Hem.	48.0	1366	**838**	**528**			
		After 1 Hem.	37.7	1231	**843**	**388**	684	379	20
		After 2 Hem.	26.0	1167	**872**	**295**	663	273	23
21–56 Table 1	12.6	Before Hem.	55.2	1173	**633**	**540**			
		After 1 Hem.	44.0	1075	**706**	**369**	493	360	25
		After 2 Hem.	31.8	1204	**899**	**305**	577	269	20
21–64 Table 2	9.8	Before Hem.	46.0	999	**653**	**346**			
		After 1 Hem.	32.7	920	**692**	**228**	515	220	25
		After 2 Hem.	26.8	960	**752**	**208**	583	163	17
19–102	16.4	Before Hem.	60.8	1291	**627**	**664**			
		After 1 Hem.	43.0	1264	**817**	**447**	472	440	28
		After 2 Hem.	31.5	1292	**944**	**348**	592	282	29
Averages......		Before Hem.	52.7	1207	**682**	**519**			
		After 1 Hem.	39.3	1122	**764**	**358**	541	349	24
		After 2 Hem.	28.8	1155	**866**	**289**	603	246	22

Hemorrhage in these experiments preceded the blood volume determinations by 18 to 22 hours.

Table 2 again is representative of many experiments. The red cell hematocrit to begin with was a little subnormal but the experiment is similar in all respects to table 1. The second hemorrhage in both experiments was less than 25 per cent of the true blood volume. We note again an increase in the true plasma volume 20 hours after each hemorrhage.

Summary table 3 gives four similar experiments including those of tables 1 and 2. It should be noted that there was no shock or prostration following these hemorrhages in any of these experiments in table 3. A few other experiments presenting a mild grade of shock have not been included here but in such experiments we have noted no differences in reaction on the part of the plasma volume or hemoglobin volume.

TABLE 4

Simultaneous blood volume determinations immediately after 2 bleedings of 18 per cent and 21 per cent of true blood volume

Dog 20–101. Airedale, young adult, female

DATE	METHOD	TOTAL BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	EXPECTED VOLUME		JUGULAR R. B. C. HEMATOCRIT	VOLUME PER KILO			TRUE BLOOD VOLUME	TRUE R. B. C. HEMATOCRIT	WEIGHT
					Plasma	Cell		Total blood volume	Plasma volume	R. B. C. volume			
1921		*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*	*kilos*
4/7 3:00 p.m.	Hgb.	2288	**1096**	1192			52.1	114.4	54.8	59.6		44.7	20.0
	CO	1535	649	**886**			57.7	76.7	32.4	44.3	**1982**		
4/11	3:00–3:05 p.m. Bled 355 cc. Hemat. 50.0 per cent. Cells 178 cc. Plasma 177 cc. Blood Vol. det. 5 minutes later												
	Hgb.	1675	**872**	803	919		48.0	91.1	47.4	43.7		41.8	18.4
	CO	1385	755	**630**		690	45.5	75.4	41.1	34.3	**1502**		
4/12	3:00–3:05 p.m. Bled 325 cc. Hemat. 37.9 per cent. Cells 123 cc. Plasma 202 cc. Blood Vol. det. 5 minutes later												
	Hgb.	1492	**918**	574	993		38.5	78.2	48.1	30.1		34.2	19.1
	CO	1324	846	**478**		492	36.1	69.4	44.4	25.0	**1396**		
4/13	Hgb.	1585	**1081**	504			31.8	83.0	56.6	26.4		28.7	19.1
	CO	1400	965	**435**			31.1	73.4	50.6	22.8	**1516**		
4/15	Hgb.	1530	**1048**	482			31.5	81.4	55.7	25.7		33.2	18.8
	CO	1608	1085	**523**			32.5	85.5	57.7	27.8	**1571**		
4/20	Hgb.	1495	**954**	541			36.2	74.4	47.5	26.9		38.4	20.1
	CO	1545	949	**596**			38.6	77.0	47.3	29.7	**1550**		

The averages of these four experiments are of much interest and the close approximation of measured and expected hemoglobin values (cell volume CO method) is significant. These values after the first hemorrhage differ by (358 − 349 = 2.6 per cent) a percentage within the 5 per cent error of the methods. After the second hemorrhage we note an increase in measured over the expected values (289 − 246 = 17 per cent) of 17 per cent which may be in part explained by emergency formation of new hemoglobin but perhaps in part by mobilization of some of the "immobilized hemoglobin."

We wish to note that all the figures for *red cell volume CO method include this 8 to 10 per cent immobilized hemoglobin.* This amount of CO combining material is obviously not circulating red cells or hemoglobin but is probably reasonably constant and reduces by a fixed amount in each instance the figures given in the various tables. We shall take this subject up more in detail in a subsequent paper.

By contrast (table 3) we note a great increase in actual *plasma volume* after each hemorrhage but more after the second bleeding. For this reason the true total blood volume shows almost the same values before and after the bleedings. In the dog therefore we have evidence that an effort is made to keep a *constant total volume* of circulating blood, compensating for loss of red corpuscles by an increase in plasma. The average figures show an increase in plasma volume from 682 cc. control to 764 cc. after the first bleeding, to 866 cc. after the second bleeding. This last indicates a total increase of plasma volume of 27 per cent above control level. Many other similar observations are recorded elsewhere in connection with a study of hemoglobin regeneration after anemia (3).

Unit hemorrhages followed immediately by blood volume determinations. This group of experiments is quite different in that the hemorrhage is followed immediately by the blood volume determinations. The bleeding occupied 5 minutes. There was a short period of 5 minutes for adjustment. The simultaneous blood volumes were then determined within 15 to 20 minutes, the plasma volume preceding the CO method.

Table 4 is characteristic of many other experiments. It shows that the measured and expected values for plasma and red cells are quite close. There was no evidence of shock. Many other similar experiments were performed but need not be tabulated. In some similar experiments the blood plasma volumes only or hemoglobin volumes only were determined. The experimental results were in accord.

Table 5 was complicated by shock after the second bleeding but this did not disturb the usual reaction of plasma dilution as indicated by the CO hematocrit. The slow clinical recovery of this dog following the bleedings probably explains the very slow regeneration of red cells

TABLE 5

Simultaneous blood volume determinations immediately after 2 bleedings of 21 per cent and 25 per cent of true blood volume

Dog 21–93. Police dog, male adult

DATE	METHOD	TOTAL BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	EXPECTED VOLUME		JUGULAR R. B. C. HEMATOCRIT	VOLUME PER KILO			TRUE BLOOD VOLUME	TRUE R. B. C. HEMATOCRIT	WEIGHT
					Plasma	Cell		Total blood volume	Plasma volume	R. B. C. volume			
1921		*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*	*kilos*
3/8 3:30 p.m.	Hgb.	1680	**783**	897			53.4	96.1	44.7	51.4		45.6	17.5
	CO	1281	622	**659**			51.4	73.2	35.6	37.6	**1442**		
3/9	12:55–1:03 p.m. Bled 315 cc. Hemat. 52.5 per cent. Cells 165 cc. Plasma 150 cc. Blood Vol. det. 5 minutes later.*												
	Hgb.	1236	**586**	650	633		52.6	73.5	34.8	38.7		48.1	16.8
	CO	1108	565	**543**		474	49.0	66.2	33.7	32.3	**1129**		
3/10	3:50–3:55 p.m. Bled 279 cc. Hemat. 41.6 per cent. Cells 116 cc. Plasma 163 cc. Blood Vol. det. 5 minutes later.*												
	Hgb.	1332	**764**	568	690		42.7	75.3	43.0	32.3		33.9	17.7
	CO	1170	778	**392**		413	33.5	66.2	44.0	22.2	**1156**		
3/11	Hgb.	1202	**830**	372			31.1	71.1	49.0	22.1		30.3	16.9
	CO	1240	879	**361**			29.1	73.4	52.0	21.4	**1191**		
3/14	Hgb.	1306	**944**	362			27.8	78.2	56.5	21.7		27.4	16.7
	CO	1267	911	**356**			28.1	75.8	54.5	21.3	**1300**		
3/18	Hgb.	1270	**921**	349			27.5	79.4	57.5	21.9		29.7	16.0
	CO	1379	989	**390**			28.3	86.2	61.8	24.4	**1311**		
4/6	Hgb.	1350	**803**	547			40.5	89.2	53.0	36.2		38.7	15.1
	CO	1295	790	**505**			39.0	85.8	52.3	33.5	**1308**		

* Dog shocked and CO method delayed 15 minutes (note red cell hematocrit): next day dog in poor condition; following days show recovery.

on subsequent days, the level remaining almost stationary for one week.

Summary table 6 shows three similar experiments in which we may compare the average figures for plasma and red cell volumes as *determined* and as *calculated* after hemorrhage. We see these figures are very close to each other and variations do not exceed 4 per cent.

SUMMARY TABLE 6

Simple hemorrhage experiments with simultaneous blood plasma and red cell volume determinations immediately after bleedings. Compare measured and expected cell and plasma volumes

DOG NUMBER	WEIGHT	TIME	JUGULAR R. B. C. HEMATOCRIT	TRUE VOLUME COMBINED METHOD	PLASMA VOLUME HEMOGLOBIN VOLUME	R. B. C. VOLUME CO METHOD	EXPECTED VOLUME		AMOUNT OF BLEEDING IN PER CENT OF BLOOD VOLUME
							Plasma	Cell	
	kgm.		*per cent*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*
17–154	23.7	Before Hem.	57.1	1936	**836**	**1100**			
		After 1 Hem.	54.0	1586	**711**	**875**	649	820	23
		After 2 Hem.	46.6	1542	**797**	**745**	680	650	28
21–93	17.5	Before Hem.	51.4	1442	**783**	**659**			
		After 1 Hem.	49.0	1129	**586**	**543**	633	474	21
		After 2 Hem.	33.5	1156	**764**	**392**	690	413	25
20–101	19.0	Before Hem.	57.7	1982	**1096**	**886**			
		After 1 Hem.	45.5	1502	**872**	**630**	919	690	18
		After 2 Hem.	36.1	1396	**918**	**478**	993	492	21
Averages......		Before Hem.	55.4	1786	**905**	**881**			
		After 1 Hem.	49.5	1405	**723**	**682**	733	661	21
		After 2 Hem.	38.7	1364	**826**	**538**	787	518	24

A few experiments (table 7) were performed on a somewhat different plan. The dog was bled as usual but this procedure was followed by a prompt *intravenous infusion* of a gum acacia glucose solution. The blood volume determinations were made a few minutes later. The *measured* and the *calculated* amounts of hemoglobin and red cells before and after this procedure are practically identical. There is a slight *increase* in the measured plasma volume over the calculated values indicating a slight tendency to make up the lost plasma during the hemorrhage period. Apparently this bleeding and the infusion of this solution does not in any way disturb the circulating blood volume.

TABLE 7

Simultaneous blood volumes done immediately after unit bleeding and infusion with glucose-acacia solution

Dog 21–5. Fox terrier, male adult

DATE	METHOD	TOTAL BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	EXPECTED VOLUME AFTER HEMORRHAGE		JUGULAR R. B. C. HEMATOCRIT	VOLUME PER KILO			TRUE BLOOD VOLUME	TRUE R. B. C. HEMATOCRIT	WEIGHT
					Plasma	Cell		Total blood volume	Plasma volume	R. B. C. volume			
1921		*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*	*kilos*
5/23 11:00 a.m.	Hgb.	708	**349**	359			50.6	109.5	54.1	55.5		40.1	6.46
	CO	449	216	**233**			52.0	69.4	33.3	36.1	**582**		
5/24	10:55 a.m. Bled 232 cc. Cells 107 cc. Plasma 125 cc. 11:10–11:20 a.m. Injected 200 cc. of 6 per cent glucose and 3 per cent gum acacia solution at 36°C. Blood volume determined 5 minutes later												
	Hgb.	634	**476**	159	424		25.0	103.2	77.4	25.8		18.8	6.14
	CO	397	287	**110**		118	27.7	64.8	46.8	18.0	**585**		

Dog 21–111. Fox terrier, male, adult

DATE	METHOD	TOTAL BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	EXPECTED VOLUME AFTER HEMORRHAGE		JUGULAR R. B. C. HEMATOCRIT	VOLUME PER KILO			TRUE BLOOD VOLUME	TRUE R. B. C. HEMATOCRIT	WEIGHT
					Plasma	Cell		Total blood volume	Plasma volume	R. B. C. volume			
5/23 1:00 p.m.	Hgb.	680	**344**	336			49.4	119.6	60.5	59.1		36.4	5.68
	CO	447	250	**197**			44.2	78.7	43.9	34.8	**541**		
5/24	1:25 p.m. Bled 230 cc. Cells 93 cc. Plasma 137 cc. 1:29 p.m. Injected 200 cc. of 6 per cent glucose and 3 per cent gum acacia solution at 36°C. 1:38 p.m. Blood volume determined												
	Hgb.	588	**473**	115	407		19.5	107.9	86.8	21.1		17.4	5.45
	CO	429	329	**100**		96	23.4	78.6	60.2	18.4	**573**		

CONCLUSION

Simultaneous determinations of plasma volume and hemoglobin red cell volume can be rapidly made in dogs without clinical disturbance.

Such determinations done *immediately* after a unit hemorrhage show a striking similarity between the *measured* and the *calculated* values. This indicates accuracy of measurement and should increase our confidence in the accuracy of these methods under the conditions of these experiments.

Determinations done immediately after a unit hemorrhage and intravenous infusion of a glucose-acacia solution show close approximation of *measured* values and *calculated* values.

Determinations done 18 to 24 hours after a unit hemorrhage show the expected values in hemoglobin or red cell values but a considerable *increase* in measured plasma volume. The normal dog after hemorrhage *increases its plasma volume* to make up almost completely for the loss of red cells. This plasma volume decreases to the normal level as the red cells are regenerated to normal.

We recognize that approximately 8 to 10 per cent of the hemoglobin measured is "immobilized hemoglobin" held in the muscles and bone marrow. This is not allowed for in our own figures and will be discussed in a subsequent paper. It is obviously not true circulating red cells or hemoglobin but is capable of diluting the inhaled CO.

Finally these experiments confirm our opinion concerning the total blood volume in the dog. In our opinion the blood volume is made up of plasma (approximately 5 per cent of body weight) and red cells (approximately 4 per cent of body weight) although the jugular vein hematocrit reads 50 per cent, an equal division between plasma and cells. The details of this argument are given elsewhere (5).

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Reprinted from THE AMERICAN JOURNAL OF PHYSIOLOGY
Vol. 61, No. 1, June, 1922

II. SIMULTANEOUS DETERMINATIONS OF PLASMA AND HEMOGLOBIN VOLUMES

Influence of Fluids by Mouth and Vigorous Exercise

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Received for publication March 11, 1922

In the preceding paper there is evidence that the dog tends to maintain a rather uniform total blood volume. When there is a considerable loss of red cells we note a compensating increase in plasma volume which again decreases as new red cells are regenerated. The increase in plasma volume may not make up the loss in red cells and return the total volume to 100 per cent but the plasma increase may amount to 25 per cent or more. How far this increase in plasma volume will continue as increasing amounts of red cells are lost we cannot say. Our experience in this respect is different from the observations of Bock (1) and others to the effect that in man the plasma volume is a constant in spite of loss of red cells. It will be of interest to supplement these casual observations by more complete studies of human cases in which venesection is to be done as a remedial measure (heart disease, eclampsia, etc.). In a large series of human cases the total blood volume before and after a measured bleeding should be directly measured.

We thought it proper to determine the blood volume values during certain periods of fluid intake and vigorous exercise. To understand the physiological fluctuations in blood volume obviously we must know what physiological activities may influence plasma or total hemoglobin volumes. It may be stated that liberal water intake by stomach does increase distinctly the plasma volume. On the contrary a concentrated sugar solution has a negative effect.

One is surprised at first to learn that vigorous but brief exercise does not modify the total plasma or hemoglobin volumes. Dogs exercised at a run for 20 minutes may lose 12 ounces or more in weight and this is a fluid loss but the blood plasma volume is held at its normal level. There is no increase in the red cell hematocrit after exercise to correspond with the familiar observations that the red count may rise after exercise (man).

Experimental observations. These experiments were done during the same period as the experiments in the preceding paper. General methods and experimental details are described above. The same standard dogs were used and these procedures caused the animals no discomfort. These experiments are uniform and the averages of many such experiments are convincing. Many incomplete experiments are not included in the tables but the experimental data are all in accord.

Table 8 gives in detail four representative experiments. The last experiment was complicated by vomiting but active diuresis was established and we have no evidence that vomiting under these conditions modifies the blood plasma volume increase. The first experiment shows a maximal increase of 400 cc. in plasma volume. Animals vary in their reaction to water drinking but in reviewing individual variations we must keep in mind the 3 to 5 per cent error of this method.

Table 9 gives a summary of eight complete experiments with water ingestion. Some of these experiments are complicated by vomiting but the reaction is not modified. The average of all experiments shows an increase in blood plasma volume from 964 cc. to 1143 cc. or 16 per cent increase. The red cell or hemoglobin volume remains practically unchanged. The average figures show a slight drop in red cell volume from 863 cc. to 846 cc. The jugular hematocrit shows a distinct fall from 53.3 per cent to 48.7 per cent.

Table 10 gives four complete experiments dealing with the ingestion of sugar solutions. The sugar solutions were usually given all at one time but in one experiment (dog 19–128) in divided doses. Glucose and cane sugar were used individually and combined and a variety of concentrations employed. The net result shows constant figures for the blood plasma and hemoglobin volumes. Vomiting does not appear to modify the reaction.

Table 11 gives a summary of seven complete experiments with ingestion of sugar solutions. The averages for all experiments are of particular interest and serve as control for the water experiments. The figures are practically identical before and after sugar ingestion.

Table 12 gives four representative complete experiments dealing with vigorous brief exercise. The dogs were exercised at a brisk run for a period indicated either on leash or behind an automobile. The dogs were panting and ready to lie down but not exhausted at the end of such periods. These dogs in the majority of instances were not ac-

TABLE 8

Water given by stomach tube. Blood and plasma volume increased

DATE	METHOD	TOTAL BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	JUGULAR R. B. C. HEMATOCRIT	VOLUME PER KILO			TRUE VOLUME COMBINED METHOD	TRUE R. B. C. HEMATOCRIT COMBINED METHOD	WEIGHT
						Total blood volume	Plasma volume	R B. C. volume			
	Dog no. 19–128. Hound, adult, male										
1920		*cc.*	*cc.*	*cc.*	*per cent*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*	*kgm.*
4/7	Hgb.	2340	**1080**	1260	53.9	82.2	37.9	44.3			
	CO	2150	968	**1182**	55.0	75.5	34.0	41.5	**2262**	**52.3**	28.5
4/8	9:00 a.m. 1000 cc. water 11:00 a.m. 500 cc. water 10:00 a.m. 1000 cc. water 11:30 a.m. 500 cc. water 10:30 a.m. 1000 cc. water 12:15 p.m. Blood volume estimated										
	Hgb.	2867	**1490**	1377	48.0	102.7	53.4	49.3			
	CO	2252	1126	**1126**	50.0	80.7	40.3	40.4	**2616**	**43.0**	27.9
	Dog no. 21–11. Collie, adult, female										
12/1	Hgb.	1935	**1054**	881	45.5	60.5	33.0	27.5			
	CO	1480	740	**740**	50.0	46.3	23.2	23.1	**1794**	**41.2**	32.0
12/2	9:15 a.m. 1000 cc. water 11:00 a.m. 500 cc. water 9:45 a.m. 500 cc. water 12:00 m. Blood volume estimated 10:30 a.m. 500 cc. water										
	Hgb.	2140	**1155**	985	46.0	67.5	36.5	31.0			
	CO	1572	839	**733**	46.6	49.6	26.5	23.1	**1888**	**38.8**	31.7
	Dog no. 21–6. Hound, young adult, male										
12/3	Hgb.	1547	**859**	688	44.5	98.5	54.7	43.8			
	CO	1132	628	**504**	44.5	72.2	40.1	32.1	**1363**	**36.9**	15.7
12/4	8:30 a.m. 750 cc. water 11:45 a.m. 750 cc. water 9:15 a.m. 500 cc. water 12:15 p.m. Blood volume estimated 10:00 a.m. 750 cc. water										
12/4	Hgb.	1475	**959**	516	35.0	95.8	62.3	33.5			
	CO	1220	766	**454**	37.2	79.2	49.7	29.5	**1413**	**32.1**	15.4

TABLE 8—*Concluded*

DATE	METHOD	TOTAL BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	JUGULAR R. B. C. HEMATOCRIT	VOLUME PER KILO: Total blood volume	VOLUME PER KILO: Plasma volume	VOLUME PER KILO: R. B. C. volume	TRUE VOLUME COMBINED METHOD	TRUE R. B. C. HEMATOCRIT COMBINED METHOD	WEIGHT
	Dog no. 19–128. Hound, adult, male										
1920		*cc.*	*cc.*	*cc.*	*per cent*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*	*kgm.*
3/24	Hgb.	2475	**1090**	1385	56.0	90.0	39.6	50.4			
	CO	1885	825	**1060**	56.3	68.6	30.0	38.6	**2150**	**49.3**	27.5
3/25	9:20 a.m. 1000 cc. water 9:50 a.m. 1000 cc. water 10:20 a.m. 1000 cc. water 10:50 a.m. 1000 cc. water 11:20 a.m. 1000 cc. water Dog vomited. Diuresis well established. 12:00 m. Blood volume estimated										
3/25	Hgb.	2440	**1187**	1253	51.4	89.0	43.3	45.7			
	CO	2080	999	**1081**	52.0	76.0	36.5	39.5	**2268**	**47.6**	27.4

SUMMARY TABLE 9

Increase in blood plasma volume due to water by mouth

DOG NUMBER	WEIGHT	BEFORE GIVING WATER: Jugular R. B. C. hematocrit	BEFORE GIVING WATER: Plasma volume hemoglobin method	BEFORE GIVING WATER: R. B. C. volume CO method	BEFORE GIVING WATER: True blood volume combined methods	AFTER GIVING WATER: Jugular R. B. C. hematocrit	AFTER GIVING WATER: Plasma volume hemoglobin method	AFTER GIVING WATER: R. B. C. volume CO method	AFTER GIVING WATER: True blood volume combined methods	WATER AMOUNT GIVEN
Uncomplicated by vomiting										
	kilos	*per cent*	*cc.*	*cc.*	*cc.*	*per cent*	*cc.*	*cc.*	*cc.*	*liters*
18–4	18.2	55.2	**865**	**722**	1587	54.1	**882**	**835**	1717	5.0
19–128	28.5	54.5	**1080**	**1182**	2262	49.0	**1490**	**1126**	2616	4.0
21–11	32.0	47.8	**1054**	**740**	1794	46.3	**1155**	**733**	1888	2.5
21–6	15.7	44.5	**859**	**504**	1363	36.1	**959**	**454**	1413	2.8
17–154	25.3	59.8	**930**	**1130**	2060	54.7	**1186**	**1052**	2238	2.5
Average.......	23.9	52.3	**957**	**855**	1812	48.0	**1134**	**840**	1974	
Complicated by vomiting but diuresis well established										
19–128	28.2	56.5	**1065**	**1029**	2094	51.7	**1410**	**924**	2334	5.0
19–128	27.5	56.2	**1090**	**1060**	2150	51.7	**1187**	**1081**	2268	5.0
21–56	14.8	50.6	**762**	**525**	1287	45.3	**859**	**552**	1411	2.5
Average.......	23.5	54.4	**972**	**871**	1843	49.5	**1152**	**852**	2004	
Average all experiments.....	23.7	53.3	**964**	**863**	1827	48.7	**1143**	**846**	1989	

TABLE 10

Sugar solution given by stomach tube. Blood plasma volume unchanged

DATE	METHOD	TOTAL BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	JUGULAR R. B. C. HEMATOCRIT	VOLUME PER KILO			TRUE VOLUME COMBINED METHOD	R. B. C. HEMATOCRIT COMBINED METHOD	WEIGHT
						Total blood volume	Plasma volume	R. B. C. volume			
Dog no. 19-128. Hound, adult, male											
1920		*cc.*	*cc.*	*cc.*	*per cent*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*	*kilos*
8/5	Dye	2610	**1262**	1348	52.6	101.6	49.1	52.5			
	CO	2093	936	**1157**	55.3	81.5	36.5	45.0	**2419**	**47.8**	25.7
8/6	10:15 a.m.	500 gm. of sucrose in 350 cc. warm water									
	1:15 p.m.	Blood volume dye method									
	2:15 p.m.	Blood volume CO method									
8/6	Dye	2500	**1244**	1256	50.3	99.3	49.4	49.9			
	CO	2078	946	**1132**	54.5	82.4	37.5	44.9	**2376**	**47.7**	25.2
Dog no. 19-128. Hound, adult, male											
1921											
5/19	Hgb.	2469	**990**	1479	59.9	96.6	38.7	57.9			
	CO	1967	744	**1223**	62.2	77.1	29.1	48.0	**2213**	**55.4**	25.5
5/13	9:30 a.m.	75 gm. glucose + 25 gm. sucrose in 800 cc. warm water									
	10:30 a.m.	75 gm. glucose + 25 gm. sucrose in 800 cc. warm water									
	11:30 a.m.	75 gm. glucose + 25 gm. sucrose in 800 cc. warm water									
	12:30 p.m.	Blood volume estimated									
5/13	Hgb.	2427	**995**	1432	59.0	95.4	39.1	56.3			
	CO	2026	810	**1216**	60.0	80.0	32.0	48.0	**2211**	**55.0**	25.4
Dog no. 18-60. Bull, old male											
1920											
6/22	Hgb.	2071	**976**	1095	52.9	86.0	40.5	45.5			
	CO	1983	972	**1011**	51.0	82.4	40.4	42.0	**1987**	**51.0**	24.1
6/23	10:30 a.m.	890 gm. of sucrose in 980 cc. of warm water									
	12:30 p.m.	Dog vomited 200 cc.									
	2:00 p.m.	Blood volume estimated									
6/23	Hgb.	2070	**915**	1155	55.8	87.8	38.8	49.0			
	CO	1866	802	**1064**	57.0	79.0	34.0	45.0	**1979**	**53.9**	23.6

TABLE 10—*Concluded*

DATE	METHOD	TOTAL BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	JUGULAR R. B. C. HEMATOCRIT	VOLUME PER KILO			TRUE VOLUME COMBINED METHOD	R. B. C. HEMATOCRIT COMBINED METHOD	WEIGHT
						Total blood volume	Plasma volume	R. B. C. volume			
Dog no. 17–160. Hound, adult, male											
1920		*cc.*	*cc.*	*cc.*	*per cent*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*	*kilos*
6/24	Hgb.	2338	**1159**	1179	50.4	96.7	48.0	48.7			
	CO	1750	818	**932**	53.2	72.4	33.9	38.5	**2091**	**44.6**	24.2
6/25	11:00 a.m.	1170 gm. of sucrose in 1300 cc. warm water									
	12:00 m.	Dog vomited 400 cc.									
	2:00 p.m.	Blood volume estimated									
6/25	Hgb.	2373	**1148**	1225	51.6	100.0	48.4	51.6			
	CO	1750	825	**925**	52.8	73.8	34.8	39.0	**2073**	**44.6**	23.7

SUMMARY TABLE 11

Blood plasma volume unchanged by ingestion of concentrated solution of sugar

DOG NUMBER	WEIGHT	BEFORE GIVING SUGAR				AFTER GIVING SUGAR				SUGAR SOLUTION	
		Jugular R. B. C. hematocrit	Plasma volume hemoglobin method	R. B. C. volume CO method	True blood volume combined methods	Jugular R. B. C. hematocrit	Plasma volume hemoglobin method	R. B. C. volume CO method	True blood volume combined methods	Sugar	Water
Uncomplicated by vomiting											
	kilos	*per cent*	*cc.*	*cc.*	*cc.*	*per cent*	*cc.*	*cc.*	*cc.*	*grams*	*cc.*
19–128	27.4	57.5	**1134**	**1152**	2286	59.6	**1052**	**1182**	2234	500	300
19–128	25.7	53.9	**1262**	**1157**	2419	52.4	**1244**	**1132**	2376	500	350
21–6	10.6	37.8	**763**	**378**	1141	36.7	**747**	**355**	1102	50	150
19–128	25.5	61.0	**990**	**1223**	2213	59.5	**995**	**1216**	2211	300	240
Average........	22.3	52.5	**1037**	**977**	2014	52.0	**1009**	**971**	1980		
Complicated by vomiting											
18–60	24.1	52.0	**976**	**1011**	1987	56.4	**915**	**1064**	1979	890	980
17–160	24.2	51.8	**1159**	**932**	2091	52.2	**1148**	**925**	2073	1170	1300
19–102	18.3	58.6	**798**	**713**	1511	58.8	**765**	**762**	1527	400	300
Average........	22.2	54.1	**977**	**885**	1863	55.8	**942**	**917**	1859		
Average all experiments......	22.2	53.3	**1007**	**931**	1938	53.9	**975**	**944**	1919		

TABLE 12

Simultaneous determinations of blood plasma and red cell volume after vigorous exercise

DATE	METHOD	TOTAL BLOOD VOLUME	PLASMA VOLUME	R. B. C. VOLUME	JUGULAR R. B. C. HEMATOCRIT	VOLUME PER KILO			TRUE VOLUME COMBINED METHOD	TRUE R. B. C. HEMATOCRIT COMBINED METHODS	WEIGHT
						Total blood volume	Plasma volume	R. B. C. volume			
	Dog no. 18–4. Sheep dog, adult, female										
1920		*cc.*	*cc.*	*cc.*	*per cent*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*	*kgm.*
4/19	Hgb. CO	2100 1710	**1080** 855	1020 **855**	48.6 50.0	114 93	58.5 46.5	55.5 46.5	 **1935**	 **44.2**	 18.4
4/20	9:30 a.m.–10:00 a.m. Dog exercised at a run on leash; well exercised and panting though not very tired 10:00 a.m.–10:20 a.m. Blood volume estimated										
4/20	Hgb. CO	1815 1754	**925** 860	890 **894**	49.0 51.0	102.5 99.1	52.3 48.6	50.2 50.5	 **1819**	 **49.1**	 17.7
	Dog no. 20–80. Hound, young adult, male										
4/22	Hgb. CO	2478 1376	**1243** 681	1235 **695**	49.9 50.5	133.7 74.4	67.0 36.8	66.7 37.6	 **1938**	 **35.9**	 18.5
4/23	11:15 a.m.–11:45 a.m. Dog exercised at run behind automobile; dog panting but not tired 11:45 a.m.–12:15 p.m. Blood volume estimated										
4/23	Hgb. CO	2060 1430	**1084** 736	976 **694**	47.4 48.5	111.2 77.3	58.6 39.8	52.6 37.5	 **1778**	 **39.0**	 18.5
	Dog no. 19–128. Hound, adult, male										
4/29 4/28	Hgb. CO	2561 1884	**1136** 731	1425 **1153**	55.6 61.2	92.9 68.3	41.3 26.5	51.6 41.8	 **2289**	 **50.4**	 27.6
4/30	11:00 a.m.–11:12 a.m. Dog exercised at a run on leash 11:15 a.m. Blood volume estimated										
4/30	Hgb. CO	2550 1943	**1190** 894	1360 **1049**	53.4 54.0	94.8 72.3	44.2 33.3	50.6 39.0	 **2239**	 **46.9**	 26.9
	Dog no. 20–101. Airedale, young adult, male										
1921 1/13	Hgb. CO	1936 1794	**968** 884	968 **910**	50.0 50.7	90.3 83.6	45.1 41.2	45.2 42.4	 **1878**	 **48.5**	 21.46
1/4	2:00 p.m. Dog exercised at a run behind car, rate 12 miles per hour for 3 miles 2:15 p.m. Blood volume estimated										
1/4	Hgb. CO	2101 1705	**1006** 817	1095 **888**	52.1 52.1	98.0 79.5	47.0 38.1	51.0 41.4	 **1894**	 **46.9**	 21.46

customed to vigorous exercise and many of them were rather fat. We have observed no difference in these experiments between the active lean dogs and the fat inactive ones although we have pointed out the fact that the active dogs possess a higher plasma volume per kilo body weight (2).

SUMMARY TABLE 13

No uniform change in blood plasma or red cell volume immediately following vigorous exercise

DOG NUMBER	WEIGHT	BEFORE EXERCISE					AFTER EXERCISE				
		Jugular R. B. C. hematocrit	Plasma volume hemoglobin method	R. B. C. volume CO method	True blood volume combined methods	R. B. C. hematocrit combined methods	Jugular R. B. C. hematocrit	Plasma volume hemoglobin method	R. B. C. volume CO method	True blood volume combined methods	R. B. C. hematocrit combined methods
	kgm.	*per cent*	*cc.*	*cc.*	*cc.*	*per cent*	*per cent*	*cc.*	*cc.*	*cc.*	*per cent*
18–4	18.4	49.3	1080	855	1935	44.2	50.0	925	894	1819	49.1
20–80	18.5	50.2	1243	695	1938	35.9	48.0	1084	694	1778	39.0
19–128	27.6	58.4	1136	1153	2289	50.4	53.7	1190	1049	2239	46.9
19–128	27.1	59.7	1147	1193	2340	51.0	61.0	882	1250	2132	58.6
19–128	26.1	47.2	1117	1020	2137	47.8	45.1	986	990	1976	50.1
17–154	23.2	57.6	995	1060	2055	51.6	59.3	1049	1102	2151	51.3
19–102	16.8	50.8	875	663	1538	43.1	43.4	865	644	1509	42.6
21–11	31.8	46.7	1075	665	1740	38.2	47.7	980	692	1672	41.4
19–102	17.2	54.8	708	610	1318	46.3	51.0	793	574	1367	42.0
20–101	22.2	58.3	906	938	1838	50.7	55.0	985	804	1789	44.9
19–102	18.1	53.7	772	661	1433	46.1	50.4	880	605	1485	40.7
20–101	21.4	50.3	968	910	1878	48.5	52.1	1006	888	1894	46.9
21–6	15.2	54.0	737	568	1305	43.5	51.2	712	556	1268	43.9
Average..	21.8	53.1	982	845	1827	45.9	49.8	950	829	1779	46.6

Table 13 gives a summary of thirteen complete exercise experiments. The exercise periods varied from 10 minutes to 30 minutes of brisk running. The majority of experiments were of 12 to 15 minutes duration and averaged about 2 to 3 miles distance. We note a slight decrease in plasma volume in the total average figures but this 3 per cent decrease is too small to be significant. The hemoglobin or red cell volume likewise shows a slight decrease in the total averages.

Discussion. Copious *water ingestion* will obviously increase the blood plasma volume,—in our experiments the average increase is 16 per cent with variations between 10 and 40 per cent. In these experiments the water was given in large amounts and a very active diuresis was established. It may be safe to assume that these increases in plasma volume are maximal but it is obvious that small amounts of water by stomach may have some influence on the plasma volume. It will be interesting to determine how long this increase in blood plasma volume persists—whether, for example, it lasts only during the period of very active diuresis. One suspects that this increase in plasma volume may be in part responsible for the great secretory activity of the kidney, but there are many other factors which must be weighed in an analysis of this problem.

Sugar solutions given by mouth exert no influence on the blood plasma volume. Concentrations of varying degree were used with similar result. We suspected the possibility that such solutions might cause a decrease in plasma volume due to intestinal fluid secretion. We also considered the possibility that the rise in blood sugar might be sufficient to call fluid from the tissues into the blood stream. The diffusion of the sugar solution in the body is evidently sufficiently rapid to prevent any such disturbance in the equilibrium of the fluids in the blood plasma. If any further method control is needed these sugar experiments furnish the data to contrast with the water drinking experiments.

The *exercise experiments* are of much interest but supply only a part of the data needed for a complete understanding of the physiology of the blood volume in exercise. These experiments are concerned with vigorous exercise periods of 10 to 30 minutes but we have no information concerning long exercise periods with or without clinical fatigue or exhaustion. When one considers the great transformation in the circulating system which must take place immediately during sudden transition from resting to active exercise or even to active digestion, it is a surprise that the blood volume is practically a constant. The blood volume remains a constant when there is a great change in the entire stream bed—for example, the active blood supply of a functioning muscle as contrasted with the resting organ. The average of thirteen complete experiments (table 13) shows a plasma volume of 982 cc. before and 950 cc. after vigorous exercise, a decrease of 3 per cent. The individual fluctuations may be almost 10 per cent. We can place no emphasis on this 3 per cent decrease in plasma volume after short exercise but if experiments of longer duration show continued plasma decrease it would be significant. There are many other points which might be discussed in connection with these experiments but we wish to reserve this discussion for a subsequent paper.

SUMMARY

Copious water ingestion will cause a distinct rise in total blood plasma volume. Our experiments show an average increase of 16 per cent.

Sugar solutions by mouth do not influence the blood plasma which remains a constant.

Short periods of vigorous exercise do not modify the blood plasma volume to a convincing degree. There are individual fluctuations but the average figures show a 3 per cent decrease after exercise. Whether this has any significance must be determined by prolonged exercise experiments.

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Reprinted from THE AMERICAN JOURNAL OF PHYSIOLOGY
Vol. 60, No. 1, March, 1922

VARIATIONS IN OUTPUT OF BILE SALTS AND PIGMENTS DURING 24-HOUR PERIODS

OBSERVATIONS ON STANDARD BILE FISTULA DOGS

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Received for publication November 19, 1921

In earlier communications from this laboratory (1), (2) have been reported many series of observations dealing with the physiology of the bile pigments and bile salts in healthy bile fistula dogs. It has been shown for example that the bile pigments can be modified at will by diet factors and many agencies which modify liver function (1). The bile pigment output therefore is varied by the constructive activity of the liver as well as by the commonly accepted disintegration of hemoglobin in the body. So too the bile acids or salts in bile fistula bile may be modified readily and promptly by diet factors (3). It is easy to demonstrate complete dissociation of these two activities of the liver showing that bile pigment production is a totally different function of the liver and separated completely from the bile salt production.

In all of our earlier experiments and as a routine we used unit daily collections of 6 hours. We felt that this period of 6 hours could be tolerated easily by a healthy dog and that the general condition of the dog was a very important element in the experiment. We have been able to maintain in perfect health many dogs on this daily routine. Some of our bile fistula animals have lived in perfect health for 2 to 4 years. We felt that the 6-hour unit collection made at the same time each day, preceded and followed by exercise, together with regular feeding hours, would be truly representative of the whole day's output. Many other workers have used 24-hour periods and we have reviewed this work elsewhere (4). In general these long collection periods tend to produce abnormalities in the dogs and the results may therefore be open to just criticism.

For our own information, we felt that it was necessary to make several 24-hour collection periods on our standard dogs. We believe these data for 24-hour experiments are more satisfactory than any available in the literature. Moreover, it enables us to correlate our published work with some of the experiments of the older investigators. The routine given below does not cause impairment of health and was carried out with much care as to detail. The dogs were not upset by the procedure and were comfortable during the entire 24-hour period. Many other experiments of similar type were carried out but are not included here as the tabulated experiments are truly representative.

Methods. The method of bile fistula collection has been described elsewhere (4). So too the operative procedure and general hygienic routine have been reviewed (4). The *daily routine* for these experiments was as follows: The regular 6-hour collection was made for the first 4 days of the week. This daily routine consisted of yard exercise followed by a preliminary drainage period of 30 minutes which allowed the escape of any excess of night bile. In some dogs there is an accumulation of bile during the night owing to slight obstruction in the fistula when the collection tube is not in place. This causes more or less dilatation of the bile passages and is not a desirable factor. The regular 6-hour collection follows this preliminary drainage. Dogs are fed 2 hours after this collection is started unless note is made to the contrary. At the end of the collection, after the collection binders have been removed, the dogs are exercised, fed and locked in their cages.

On the fifth day of the week, the dogs were put through the regular routine, except that at 4 p.m., instead of taking off the binders, removing the drainage tubes and putting the dogs away in their cages for the night, a second collection period was started. This second period lasted from 4:00 to 10:00 p.m. At about 4:00 p.m., the dogs were transferred to their cages and were kept in an upright position by specially prepared night binders. These consisted of canvas slings suspended from the tops of the cages and provided with four holes for the dog's legs and an additional hole for the drainage tube. These binders were suspended at such a height that the dogs could either stand up, or rest the weight of their bodies on the binders by a slight flexion of their legs. Rests were provided for the dogs' heads, so that they could sleep comfortably in a semi-standing position. A third collection period extended from 10 p.m. to 4 a.m. on the following day and a fourth period lasted from 4 a.m. to 10 a.m., thus completing the 24-hour drainage and securing the day's output of bile in four 6-hour samples. The weekly routine thus consisted of a single 6-hour collection on the first four days of the week, followed by four consecutive 6-hour collections on the fifth day. The dogs were then rested till the following Monday.

Analysis of bile. The specimens of bile secured were analyzed for their content of bile acids and bile pigments. These methods have been fully described elsewhere in the publications of this laboratory. In brief, the bile acid method (5) consists in precipitation of proteins by alcohol, followed by an estimation of amino nitrogen present in the unhydrolyzed sample. A sample of the alcoholic filtrate is then hydrolyzed by means of sodium hydrate and its content of amino nitrogen estimated. This, minus the amount of amino nitrogen present in the unhydrolyzed specimen represents the amino nitrogen held in combination as taurocholic acid. The amount of taurocholic acid is estimated from the amino nitrogen by multiplying by the factor 36.72. Taurocholic acid is the only bile acid found in dog's bile.

The bile pigment method consists, in brief, of the following procedure. A 1 to 50 or weaker dilution of the bile in acid alcohol is filtered and allowed to stand in a flask for 24 hours, or until the pigment is converted to a blue green color. The solution is then read against a standard color wedge (4). The pigment readings are all expressed in terms of milligrams of bilirubin.

Feeding of dogs. The diet of the dogs, on the day of the experiments, was carefully controlled. At night the animals were always kept in metabolism cages, with only water before them. In the day time all food was excluded except the particular diet upon which the dogs were being kept, and they were fed at stated times twice a day. The amounts of food ingested were regularly recorded. Over the week-ends the dogs were rested on a mixed diet.

The carbohydrate diet referred to in the tables consisted of a mixture of potatoes, rice and milk, in the following proportions: potatoes, 40 per cent; rice, 28 per cent; milk, 32 per cent.

The meat diet consisted of meat alone. On most occasions extracted veal, obtained from the media kitchen, was used; but occasionally scraps of roast beef from the hospital kitchen were fed. On the days when mixed diet was used, the amounts ingested were not recorded. The mixed diet of course varied in content, but usually consisted of a mixture of bread and milk, table scraps and varying amounts of meat.

Experimental observations. These observations were made on three bile fistula dogs of very different type and are representative of the reaction of normal dogs under these conditions. One dog (20-99) was a very active, young, white bull dog, nervous and excitable but raised in the laboratory kennels and undisturbed by the laboratory routine. This dog had a strong habit spasm of its shoulder muscles. Bile was completely excluded from the duodenum as shown by autopsy. Another dog (18-123) was a young adult mongrel, active and lively but good natured and not disturbed by the laboratory routine. Bile was completely excluded from the duodenum as shown by autopsy. The third dog (18-30) was a very strong, vigorous and rather vicious female of about 6 years of age. She was in perfect physical condition during the 2 years of experimental work. Her appetite was capricious and she refused many food mixtures. At operation the bile fistula was made as usual but the common duct only ligated, not cut. In due time, a small communicating channel was reëstablished permitting the flow of a little bile into the duodenum when external drainage was not in effect (at night and outside of collection periods). Autopsy showed the condition as described and recognized clinically.

Descriptions of bile fistula dogs. Following are the records of the essential points in the life histories of the dogs used in this work; also certain post-mortem findings.

Dog 20–99. White bull, young male. Weight on March 26, 1920, 32.5 pounds. Dog has marked nervous tic of both fore limbs. Operation on March 26, 1920—common duct cut between ligatures; fistula made as usual. Recovery speedy and uneventful. Dog set up on April 5th for daily bile collections. Used in bile metabolism work from this time until January 29, 1921. Stools clay colored. January 26, 1921, blood serum quite icteric. Red cell hematocrit, 38 per cent. On January 31st, dog was observed to be very feeble and collections of bile were discontinued. Dog went rapidly downhill and on February 8th began to bleed from his fistula. Dog bled all night and in morning red cell hematocrit was 33 per cent, serum being decidedly icteric. Dog given excess of chloroform and immediately autopsied.

Autopsy findings: Remains of an emaciated dog (weight 20 lbs.). Fistula appears normal externally, but a bloody bile is exuding from it. Gums and other mucous membranes are extremely anemic. Median incision reveals normal subcutaneous tissues and muscles. There are a few fibrous adhesions between the omentum and operative site. The intestine and pancreas are stained a dark, maple sugar color. The costo-chondral junctures are enlarged. The ribs are very fragile and almost completely destitute of lime salts. Almost all the ribs show fusiform enlargements where spontaneous fractures with subsequent healing have occurred. The flat bones show the same process. The long bones of the extremities seem normal and contain normal marrow. The bones of the skull show some diminution of lime salts.

Heart and lungs—negative. Thyroid—small and pale. Spleen—negative.

Liver appears normal. No cirrhotic thickening. Slight scarring on a portion of ventral surface. Gall passages—hepatic and cystic ducts are patent and moderately dilated. Common duct is completely occluded and ends in a blind pouch.

Pancreas—deep, maple sugar color. Negative, on section. Duct opens normally into duodenum. Stomach and intestines—negative except for brown color of muscle coats. Kidneys and bladder and prostate—negative. Adrenals—negative. Lymph nodes—a few are enlarged in region of liver hilus, also a few of the mesenteric nodes are enlarged. Brain appears negative superficially.

Microscopical sections: Liver—slight degree of round cell infiltration around some of smallest ducts. No cirrhosis. Moderate degree of brown atrophy. Central cells pale and contain brown pigment. A study of the bone abnormalities will be reserved for a future communication.

Dog 18-30. White bull, middle age, female. Weight on August 19, 1919, 39 pounds. Operation on August 19, 1919. Three ligatures placed on duct, but duct not cut. Fistula as usual. Recovery uneventful. Dog set up on September 10th. November, 1920, feces gave positive test for stercobilin with Schlesinger's reagent, indicating that exclusion of bile from duodenum is incomplete. Dog keeps her weight well. June 15, 1921, dog normal. Chloroform anesthesia and killed; autopsy performed at once.

Autopsy findings: Heart—normal. Lungs—slight anthracosis, normal on section. Spleen—rather large, firm and fibrous on section.

Liver—normal size, red-brown in color with occasional yellowish tinge; lobulation normal on section. Gall bladder attached to abdominal wall by way of operative fistula. A fine probe can be passed from papilla into common duct. Site of operation shows a definite stricture leaving an aperture not over 1 mm. in diameter. A slight dilatation exists above this. Probably drainage into duodenum was pretty efficient at night.

Stomach and intestines—show congestion due to a toxic proteose administered a few days previously. Pancreas—quite firm; section normal. Kidneys—fairly large in size, capsule adherent; section normal. Bladder—normal. Skeletal muscle—normal; slight amount of fat. Bones—apparently no thinning and no loss of lime salts. Bone marrow—no hyperplasia; normal in appearance.

Dog 18-123. White and tan, adult, female. Weight on October 9, 1919, 25 pounds. October 9th, exposure to x-rays over spleen region (200 milliampere minutes at distance of 10 inches from skin). Operation on October 21, 1919. Weight 26 pounds. Common duct cut and ligated above and below. Fistula as usual. Recovery uneventful. Dog set up on October 31st. Stools clay colored. Used for daily collections from this time until March 8, 1921, when she became intoxicated. Died March 10th.

Autopsy findings: Remains of an emaciated dog. Fistula externally appears in normal condition. Abdomen is moderately distended. Median incision reveals lack of subcutaneous fat. The ribs are quite fragile, containing a very small amount of lime salts. They bear numerous greyish enlargements, at the sites of previously healed fractures. The lower end of the sternum and the adjacent costal cartilages are bulged forward.

Heart and lungs—negative. Liver—a few small scars are present over the surface of the organ. It is negative on section. The larger ducts are much distended. Common duct is completely occluded at operative site. Spleen—small (12 × 3 × 0.5 cm.); weight—11 grams. On section, the fibrous elements are seen to be increased in amount. Kidneys—pale in color; capsules strip readily. Over the surfaces are a few small retention cysts. Pelves—negative. Adrenals—negative. Pancreas—brown in color; negative, on section. Intestines—brown color in muscle coats, moderate distention with gas.

Microscopical sections: Liver—central cells of lobules show beginning disintegration. Their nuclei show some degree of pyknosis, while cytoplasm is very pale and reticulated. Some of these cells contain masses of brown material. Peripheral cells of lobule—normal in appearance. No cirrhotic changes. No cholangitis. Spleen—capsule and trabeculae thickened. Malpighian bodies normal. Sinusoids widely patent and with red cells in them. Pulp cells appear normal. There is probably a slight relative decrease in parenchyma cells.

Table 1 gives three similar experimental periods on the same dog. This bile fistula animal had been under observation on the standard laboratory routine for about 13 months. The only complicating detail was an exposure of the splenic area to a considerable x-ray dose 2 weeks before the fistula operation. This dog shows the fluctuation in bile salts which are observed and so far are unexplained. We note the rise in bile salts following a change from a carbohydrate to a meat diet in the first and second experiments. This certainly is a physiological constant. The 24-hour collections show a moderately uniform series. There are fluctuations as are observed in the daily 6-hour periods but in general the four periods of 6 hours each in the 24-hour unit show uniform figures for volume and bile salts. We can detect no difference in this dog between the day and night collection periods. The bile pigment figures in this dog are high—in fact, more than twice normal and we observe some remarkable fluctuation in values. We cannot explain these reactions but they are more frequent in dogs with splenectomy as reported elsewhere (6).

Table 2 shows two experimental periods on the same dog. We note no difference during the night and day periods of the 24-hour collections. The general picture is very much like that noted in table 1.

Table 3 shows two more meat feeding experiments on the third dog of this series. The general type of reaction is the same as in the other two dogs. The rise in bile acids when the dog changes from a carbohydrate to a meat diet is very pronounced and probably more conspicuous in this dog which always ate sparingly of the carbohydrate mixtures but largely of the meat diet. During the second 24-hour period, the dog struggled very vigorously in an attempt to get out of the binder, but to no effect. There is a rise in bile volume flow but little other change to be attributed to this over-activity. As a rule during the night and much of the day, this dog dozed comfortably in its binder. There are very great fluctuations in bile pigment values.

Table 4 shows three collection periods on the same dog on a carbohydrate diet. There is a fairly uniform level of bile acid excretion

TABLE 1

Twenty-four hour bile collections after meat feeding

Dog 18–123. Brindle, mongrel, adult, female

DATE, 1920		VOLUME	AMINO NITROGEN		TAUROCHOLIC ACID IN 6 HOURS	BILIRUBIN IN 6 HOURS	WEIGHT	REMARKS
			In 1 cc. bile	Total in 6 hours				
		cc.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*lbs.*	
9/27		26	0.266	6.92	254	50.6	18.0	Mixed diet
9/28		30	0.269	8.07	296	32.9	19.0	Carbohydrate diet
9/29		38	0.324	12.31	452	25.2	18.8	Carbohydrate diet
9/30		32	0.352	11.26	413	37.8	18.0	Beef scraps (430 gm.)
10/1	a	40	0.390	15.60	572	50.2	18.3	Beef scraps (1000 gm.) 10 a.m.–4 p.m.
	b	43	0.321	13.07	479	61.0		Dog vomited mucus 4 p.m.–10 p.m.
10/2	c	38	0.460	17.48	642	40.3		Dog vomited mucus 10 p.m.–4 a.m.
	d	45	0.348	15.66	574	51.7		4 a.m.–10 a.m.
11/15		29	0.112	3.25	119	37.9	17.5	Carbohydrate diet (1000 gm.)
11/16		22	0.180	3.96	145	25.3	17.5	Carbohydrate diet (500 gm.)
11/17		32	0.318	10.18	374	33.4	17.0	Meat diet (600 gm.)
11/18	a	49	0.443	21.71	797	32.8	17.0	Meat diet (475 gm.) 10 a.m.–4 p.m.
	b	37	0.255	9.43	344	15.7		4 p.m.–10 p.m.
11/19	c	40	0.398	15.92	584	17.9		10 p.m.–4 a.m.
	d	21	0.417	18.76	688	98.6		4 a.m.–10 a.m.
11/29		35	0.439	15.36	564	42.4	18.5	Carbohydrate diet (870 gm.)
11/30		28	0.595	16.66	611	29.7	17.5	Carbohydrate diet (700 gm.)
12/1		27	0.308	8.31	303	30.6	17.0	Meat diet (350 gm.)
12/2		32	0.397	12.70	466	54.7	16.0	Meat diet (600 gm.)
12/3	a	45	0.496	22.32	818	45.4	16.5	Meat diet (425 gm.) 10 a.m.–4 p.m.
	b	45	0.368	16.56	607	39.5		4 p.m.–10 p.m.
12/4	c							10 p.m.–4 a.m.
	d	27	0.410	11.07	406	45.1		4 a.m.–10 a.m.

during most of the collection periods. In two of the observation periods there is a fall in bile salt output in the early morning hours. This may or may not be associated with a fall in volume output. The bile pigment output is very irregular and periods of semi-obstruction in the bile passages may have been responsible.

TABLE 2

Twenty-four hour bile collections after meat feeding

Dog 20–99. White bull dog, young, adult male

DATE, 1920	VOLUME	AMINO NITROGEN		TAUROCHOLIC ACID IN 6 HOURS	BILIRUBIN IN 6 HOURS	WEIGHT	REMARKS
		In 1 cc. bile	Total in 6 hours				
	cc.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*lbs.*	
11/29	37	0.411	15.21	558	56.7	28.0	Carbohydrate diet (975 gm.)
11/30	46	0.170	7.82	286	54.0	26.0	Carbohydrate diet (700 gm.)
12/1	35	0.280	9.80	358	34.1	25.5	Meat diet (800 gm.)
12/2	50	0.326	16.30	598	22.1	25.0	Meat diet (600 gm.)
12/3 a	59	0.354	20.89	766	36.3	24.8	Meat diet (450 gm.) 10 a.m.–4 p.m.
b	50	0.283	14.15	519	47.0		4 p.m.–10 p.m.
12/4 c	48	0.198	9.50	349	63.0		10 p.m.–7 a.m.
d	40	0.382	15.28	561	33.4		7 a.m.-1 p.m.
11/15	30	0.267	8.01	292	35.5	26.0	Carbohydrate diet (1000 gm.)
11/16	51	0.194	9.89	361	65.0	26.0	Carbohydrate diet (500 gm.)
11/17	53	0.165	8.74	319	25.8	25.8	Meat diet (600 gm.)
11/18							Meat diet
11/19 a	25	0.318	7.95	290		26.0	Meat diet (475 gm.) 10 a.m.–4 p.m.
b	21	0.141	2.96	108			4 p.m.–10 p.m.
11/20 c	38	0.142	5.40	197			10 p.m.–4 a.m.
d	36	0.172	6.20	226			4 a.m.–10 a.m.

Table 5 shows a series of three observations on another dog receiving a carbohydrate diet. This dog shows a fairly uniform output of bile pigment. The bile salt output is not decreased during the night hours.

Table 6 is of considerable interest and shows a dog with incomplete bile fistula on a carbohydrate diet. At night, when this dog's fistula was closed, there could flow into the duodenum a certain amount of bile. This bile influx increased the bile acid flow of the following morning. This fact, established at autopsy, we believe explains at least in part the fall in bile salts noted in both 24-hour periods. This fall in bile salts is progressive after the first 6-hour collection and reaches its lowest level in the fourth collection period in the 24-hour experiment. The reaction therefore is much like that following an ingestion of bile in the early morning (refer to table 7). The bile pigment output is high but pretty uniform. The volume output in this dog is constantly subnormal as compared with the average animal.

TABLE 3

Twenty-four hour bile collections after meat feeding

Dog 18–30. Bull dog, adult, female

DATE, 1920	VOLUME	AMINO NITROGEN		TAUROCHOLIC ACID IN 6 HOURS	BILIRUBIN IN 6 HOURS	WEIGHT	REMARKS
		In 1 cc. bile	Total in 6 hours.				
	cc.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*lbs.*	
11/15	15	0.448	6.72	247	87.7	34.0	Carbohydrate diet (170 gm.)
11/16	11	0.638	7.02	258	62.0	33.5	Carbohydrate diet (225 gm.)
11/17	17	0.971	16.51	606	85.3	33.5	Meat diet (600 gm.)
11/18 a	26	0.830	21.58	792	99.1	34.0	Meat diet (475 gm.) 10 a.m.–4 p.m.
b	9	0.770	6.93	254	10.7		4 p.m.–10 p.m.
11/19 c	11	0.908	9.99	367	9.2		10 p.m.–4 a.m.
d	17	0.948	16.12	592	23.3		4 a.m.–10 a.m.
11/29	9	1.500	13.50	495	72.4	36.0	Carbohydrate diet (450 gm.)
11/30	8	1.120	8.95	329	102.0	34.5	Carbohydrate diet (450 gm.)
12/1	16	0.644	10.30	378	116.8	35.0	Meat diet (800 gm.)
12/2	30	0.738	22.14	810	41.8	35.0	Meat diet (600 gm.)
12/3 a	15	0.850	12.75	468	43.3	35.0	Meat diet (450 gm.) 10 a.m.–4 p.m.
b	32	0.549	17.57	644	21.2		Dog struggling 4 p.m.–10 p.m.
12/4 c	22	0.595	13.09	480	16.9		10 p.m.–4 a.m.
d	17	0.877	14.91	547	111.3		4 a.m.–10 a.m.

Table 7 gives the results of three experiments on different bile fistula dogs, given large doses of taurocholic acid (2 grams) in solution by stomach. The reaction is remarkably uniform in these three different animals. The great cholagogue action does not modify the bile pigment

TABLE 4

Twenty-four hour bile collections after carbohydrate feeding

Dog 20–99. White bull dog, adult, male

DATE, 1920	VOLUME	AMINO NITROGEN		TAUROCHOLIC ACID IN 6 HOURS	BILIRUBIN IN 6 HOURS	WEIGHT	REMARKS
		In 1 cc. bile	Total in 6 hours				
	cc.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*lbs.*	
10/11	36	0.253	9.11	333	25.1	27.0	Mixed diet
10/12	44	0.283	12.45	457	21.4	26.3	Mixed diet
10/13	11	0.224	2.46	90	2.9	26.8	Carbohydrate diet (650 gm.)
10/14	63	0.112	7.05	258	20.5	25.5	Carbohydrate diet (1000 gm.)
10/15 a	23	0.140	3.22	118	15.1	25.0	Carbohydrate diet (1000 gm.)
							10 a.m.–4 p.m.
b	54	0.211	11.39	418	30.1		4 p.m.–10 p.m.
10/16 c	17	0.407	6.92	253	21.3		10 p.m.–4 a.m.
d	21	0.309	6.49	237	31.4		4 a.m.–10 a.m.
11/8	47	0.438	20.58	755	58.9	27.8	Mixed diet (1150 gm.)
11/9	57	0.397	22.62	830	117.0	27.5	Carbohydrate diet (1000 gm.)
11/10	45	0.295	13.27	487	88.5	27.3	Carbohydrate diet (1000 gm.)
11/11	49	0.337	16.50	605	112.7		Carbohydrate diet (1000 gm.)
11/12 a	38	0.308	11.70	429	83.2	26.3	Carbohydrate diet (700 gm.)
							10 a.m.–4 p.m.
b	30	0.322	9.66	353	61.6		4 p.m.–10 p.m.
11/13 c	36	0.280	10.08	370	72.7		10 p.m.–4 a.m.
d	27	0.364	9.82	355	72.4		4 a.m.–10 a.m.
12/6	55	0.255	14.02	514	15.3	24.5	Carbohydrate diet (1000 gm.)
12/7	40	0.312	12.48	457	62.4	24.5	Carbohydrate diet (700 gm.)
12/8	40	0.326	13.04	478	89.8	24.0	Carbohydrate diet (1000 gm.)
12/9	41	0.252	10.33	379	57.0	23.5	Carbohydrate diet (1000 gm.)
12/10 a	38	0.227	8.62	314	50.8	23.5	Carbohydrate diet (1000 gm.)
							10 a.m.–4 p.m.
b	27	0.241	6.51	238	38.8		4 p.m.–10 p.m.
12/11 c	37	0.113	4.18	153	40.3		10 p.m.–4 a.m.
d	28	0.284	7.95	290	53.0		4 a.m.–10 a.m.

TABLE 5

Twenty-four hour bile collections after carbohydrate feeding

Dog 18–123. Brindle mongrel, adult, female

DATE, 1920	VOLUME	AMINO NITROGEN		TAUROCHOLIC ACID IN 6 HOURS	BILIRUBIN IN 6 HOURS	WEIGHT	REMARKS
		In 1 cc. bile	Total in 6 hours				
	cc.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*lbs.*	
10/11	31	0.352	10.91	400	41.4	18.0	Mixed diet
10/12	26	0.297	7.72	282	22.6	17.0	Mixed diet
10/13	48	0.322	15.45	568	20.9	18.0	Carbohydrate diet (620 gm.)
10/14	32	0.154	4.93	180	24.5	17.0	Carbohydrate diet (830 gm.)
10/15 a	18	0.267	4.81	176	27.2	16.5	Carbohydrate diet (710 gm.) 10 a.m.–4 p.m.
10/15 b	40	0.168	6.72	245	27.8		4 p.m.–10 p.m.
10/16 c	14	0.225	3.15	115	15.3		10 p.m.–4 a.m.
10/16 d	5				6.1		4 a.m.–10 a.m.
11/8	29	0.297	8.61	314	47.4	17.0	Mixed diet
11/9	23	0.340	7.82	286	36.0	17.0	Carbohydrate diet (750 gm.)
11/10	30	0.295	8.86	324	33.7	17.5	Carbohydrate diet (1000 gm.)
11/11	38	0.309	11.75	432	32.2		Carbohydrate diet (860 gm.)
11/12 a	36	0.280	10.08	370	34.3	17.5	Carbohydrate diet (650 gm.) 10 a.m.–4 p.m.
11/12 b							4 p.m.–10 p.m.
11/13 c	23	0.322	7.41	271	50.0		10 p.m.–4 a.m.
11/13 d	22	0.392	8.62	351	53.6		4 a.m.–10 a.m.
12/6	36	0.255	9.18	337	117.9	16.3	Carbohydrate diet (800 gm.)
12/7	25	0.071	1.77	65	37.0	16.5	Carbohydrate diet (675 gm.)
12/8	23	0.298	6.85	251	36.0	16.5	Carbohydrate diet (730 gm.)
12/9	26	0.098	2.54	93	36.2	16.5	Carbohydrate diet (800 gm.)
12/10 a	24	0.156	3.74	137	25.5	16.5	Carbohydrate diet (1000 gm.) 10 a.m.–4 p.m.
12/10 b	36	0.156	5.61	206	35.1		4 p.m.–10 p.m.
12/11 c	21	0.129	2.81	103	22.3		10 p.m.–4 a.m.
12/11 d							4 a.m.–10 a.m.

output. The greater part of the 2 grams of taurocholic acid is eliminated in the 6 hours following its ingestion. In spite of this, the cholagogue action is much in evidence in the second period of 6 hours. This period of cholagogue action without any excess of bile salt excretion shows that the outpouring of an excess of fluid in bile is not a simple reaction to preserve a certain salt concentration in the bile passages.

TABLE 6

Twenty-four hour bile collections after carbohydrate feeding

Dog 18–30. Bull dog, adult, female

DATE, 1920	VOLUME	AMINO NITROGEN		TAUROCHOLIC ACID IN 6 HOURS	BILIRUBIN IN 6 HOURS	WEIGHT	REMARKS
		In 1 cc. bile	Total in 6 hours				
	cc.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*lbs.*	
11/8	6	1.170	7.02	257	51.2	35.8	Mixed diet (800 gm.)
11/9	8	0.964	7.71	283	69.6	35.0	Carbohydrate diet (70 gm.)
11/10	4	1.475	5.90	216		34.5	Carbohydrate diet (210 gm.)
11/11	10	0.857	8.57	314	100.0	34.0	Carbohydrate diet (230 gm.)
11/12 a	9	1.025	9.22	338	98.3	33.5	Carbohydrate diet (220 gm.) 10 a.m.–4 p.m.
11/12 b	7	0.616	4.31	158	85.1		4 p.m.–10 p.m.
11/13 c	10	0.364	3.64	134	96.0		10 p.m.–4 a.m.
11/13 d	6	0.420	2.52	92	71.9		4 a.m.–10 a.m.
12/6						35.0	Carbohydrate diet (50 gm.)
12/7	8	1.690	13.52	496	84.6	34.5	Carbohydrate diet (200 gm.)
12/8	10	1.090	10.90	400	98.3	34.0	Carbohydrate diet (370 gm.)
12/9	11	0.882	9.70	356	110.3	33.5	Carbohydrate diet (475 gm.)
12/10 a	22	0.383	8.43	309	51.3		Carbohydrate diet (490 gm.) 10 a.m.–4 p.m.
12/10 b	17	0.412	7.00	257	76.2		4 p.m.–10 p.m.
12/11 c	5	0.925	4.62	169	88.4		10 p.m.–4 a.m.
12/11 d	5	0.570	2.85	105	146.0		4 a.m.–10 a.m.

This emphasizes the ease with which the various elements of bile secretion may be dissociated—the fluid and salt elements and bile pigments. This all speaks for a remarkable diversity in functional activity of the liver cell. It may be well to note that this crude solution of taurocholic acid was obtained from whole dog's bile evaporated with charcoal to a soft paste, extracted with water and filtered. Obviously other elements besides taurocholic acid are present.

TABLE 7

Twenty-four hour bile collections after bile feeding

DATE, 1920	VOLUME	AMINO NITROGEN		TAUROCHOLIC ACID IN 6 HOURS	BILIRUBIN IN 6 HOURS	WEIGHT	REMARKS
		In 1 cc. bile	Total in 6 hours				
			Dog 18–123. Brindle, mongrel, adult, female				
	cc.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*lbs.*	
12/13	38	0.410	15.58	572	40.7	16.5	Mixed diet
12/14	38	0.314	11.93	440	33.3	16.5	Meat diet
12/15	40	0.422	16.88	620	34.8	17.0	Meat diet
12/16	58	0.434	25.17	924	33.7	17.0	Mixed diet
12/17 a	27	0.412	11.12	408	26.9	16.8	Mixed diet 10 a.m.–4 p.m.
b	70	0.909	63.63	2335	18.3		*4 p.m.- 10 p.m.
12/18 c	36	0.369	13.88	509	22.7		10 p.m.–4 a.m.
d	18	0.468	8.42	309	24.7		4 a.m.–10 a.m.
			Dog 18–30. Bull dog, adult, female				
12/13	13	0.452	5.87	215	61.5	33.5	Mixed diet
12/14	16	0.814	13.02	478	80.2	34.0	Mixed diet
12/15	13	1.100	14.30	525		34.0	Mixed diet
12/16	14	1.428	19.99	733	61.5	33.5	Mixed diet
12/17 a	7	0.800	5.60	206	43.9	33.3	Mixed diet 10 a.m.–4 p.m.
b	60	1.250	75.00	2755	67.2		*4 p.m.–10 p.m.
12/18 c	11	1.010	11.11	408	46.0		10 p.m.–4 a.m.
d	8	1.190	9.52	349	52.3		4 a.m.–10 a.m.
			Dog 20–99 White bull dog, young adult, male				
12/13	40	0.254	10.16	373	33.4	23.5	Mixed diet
12/14	48	0.285	13.68	502	28.4	24.0	Mixed diet
12/15	50	0.183	9.15	334	25.0	24.0	Mixed diet
12/16	50	0.238	11.90	437	41.8	23.5	Mixed diet
12/17 a	32	0.337	10.78	396	41.8	23.3	Mixed diet 10 a.m.–4 p.m.
b	67	0.955	63.98	2347	36.5		*4 p.m.–10 p.m.
12/18 c	63	0.211	13.29	487	35.6		10 p.m.–4 a.m.
d	45	0.211	9.45	345	32.9		4 a.m.–10 a.m.

* At 4 p.m., a solution of extracted dog's bile, containing 2 grams of taurocholic acid, made up to 400 cc. with water, was given by stomach tube.

DISCUSSION

In all experiments with bile fistula dogs we believe it essential to work with animals of two types—one with complete exclusion of bile from the duodenum and one with incomplete exclusion or a little seepage of bile through a small strictured common duct. This second type is well illustrated above (dog 18-30) and is of much importance. These dogs during drainage periods will certainly yield a complete bile flow as the flow through the stricture is not easy and in the standing posture the escape of bile into the cannula is very prompt and complete. But during resting or night periods there is a flow of an undetermined volume of bile into the duodenum and this is sufficient to maintain these dogs in perfect physical condition. There are certain objections to both types of experimental animal but a combination of these types gives data which we believe are trustworthy and of real physiological significance.

Dogs with biliary fistulas and complete exclusion of bile from the duodenum suffer from a variety of peculiar intoxications and metabolic disturbances, some of which have been noted previously. We record certain bony changes in the autopsy notes of two of the bile fistula dogs. (Dogs 20-99 and 18-123.) These abnormalities are of considerable interest as relating to the metabolism of inorganic salts and will be the subject of a future communication.

Our experiments with the long 24-hour collections give no constant results to indicate a decided fall in output at night or during the early morning hours. The amounts of bile acids and bile pigments are not uniformly decreased during the periods of sleep. The same statement applies to volume output of bile.

We wish again to point out the ease with which one can dissociate the various constituents of the bile (table 7). Large doses of crude taurocholic acid cause a prompt rise in bile salt excretion so that practically all the taurocholic acid is excreted within 6 hours after its ingestion. But the cholagogue action persists during the second 6-hour period or longer, showing dissociation of salt and fluid elimination. The bile pigment output remains constant throughout.

One can detect no difference in cholagogue action whether the bile salt is given during the morning or evening. The reaction will be the same during the day or during night periods when the dog is sleeping comfortably most of the time.

SUMMARY

Bile fistula dogs will show little if any difference in the output of bile, bile salts or bile pigments during four consecutive 6-hour periods. There are no constant differences to be made out. If anything can be said, it is to the effect that at times the night output is slightly less than the normal daily output. This applies to healthy bile fistula dogs.

Peculiar bony abnormalities are noted as related to the constant loss of bile in such animals.

Complete dissociation of the bile constituents can be demonstrated (table 7) after a large dose of taurocholic acid. Following a 2-gram dose we observe a great rise in bile salt content during the first 6-hour period. During the second 6-hour period the bile salt content is about normal but the cholagogue action is still much in evidence and the fluid output therefore is dissociated from the bile salts. The bile pigments are constant before and after the period of bile salt administration and elimination.

This all speaks for a remarkable diversity in liver cell function—a point not too frequently emphasized.

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Reprinted from The Journal of Biological Chemistry
Vol. L, No. 2, February, 1922

THE PHYSIOLOGY OF THE PHENOLS.

I. A QUANTITATIVE METHOD FOR THE DETERMINATION OF PHENOLS IN THE BLOOD.

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(Received for publication, December 12, 1921.)

Liver function and related problems have interested several workers in this laboratory during the past few years. As a part of this research program it seemed advisable to examine critically the conjugation of phenolic substances in the body. The mechanism of absorption, conjugation, distribution, and excretion can be studied when a suitable method is at hand. The method described and controlled below is sufficiently accurate to make possible a careful study of these phenolic substances in the blood and body tissues.

Benedict and Theis (1) in 1918 made an attempt to apply the urinary phenol method of Folin and Denis (3) to the blood. The principal modification consisted in a separate determination of uric acid by Benedict's method and an addition of sodium bisulfite to stabilize the color. Their conclusion from an examination of a number of pathological cases was that blood contains an average of 4.70 mg. of phenol per 100 cc., that it contains no conjugated phenols, and that the polyphenols appear to represent about one-third of the total phenols.

From all the experimental evidence presented by other workers we must regard as an established fact that the phenols are formed in the intestinal tract by bacterial decomposition of tyrosine, and that they are excreted largely in the urine. Only a small percentage is excreted with the feces (4). Also, it has been conclusively shown that a large percentage of the urinary phenols exists in the conjugated form (9). Since we have no reason to believe that conjugation occurs either in the kidneys or in the bladder we may safely assume that at some time or other the conjugated phenols pass through the blood prior to excretion. It should, therefore, be possible to demonstrate and measure these substances in the blood stream.

An examination of Benedict and Theis' method shows that there are several factors which may well prevent the demonstration of minute quantities of conjugated phenols. The separate determination of uric acid not only complicates the method considerably, but involves an unavoidable experimental error. The boiling of the blood filtrate down to less than half the volume is the process primarily responsible for their inability to demonstrate conjugated phenols in the blood. Benedict and Theis state that only 85 per cent of resorcinol added to the blood is recovered by their method and that phenol itself added to the blood disappears completely during the boiling of the filtrates. There are sufficient reasons to believe (9) that the bulk of the conjugated phenols is made up of two very volatile phenols—*p*-cresol and phenol—and it is, therefore, logical to assume that the boiling of the filtrates in Benedict and Theis' method is responsible for the disappearance of the conjugated phenols.

However, it is possible to modify Folin and Denis' original method so that it may be applied to blood. It seemed important to eliminate heat as a factor in the precipitation of the blood proteins and in the concentration of the filtrate. The precipitation of proteins with tungstic acid, as described by Folin and Wu (5) is an excellent substitute for the boiling acetic acid; the precipitation is fully as complete, and the reaction takes place at room temperature. In order to remove the last traces of protein, aluminum cream is added. It was further found that with a standard of 5 mg. of phenol per 100 cc. set at 20 in the Duboscq colorimeter, the color developed in the blood filtrate is easily and accurately readable. This eliminates the concentration of the filtrate by boiling as carried out by Benedict and Theis. Another objection to Benedict's method is the separate determination of uric acid by an entirely different and time-consuming method. In order to simplify this step a number of uric acid precipitants, such as Morris' (8) zinc salt and Curtman and Lehrman's (2) nickel salt were tried, but, while the precipitation of uric acid is complete, the excess zinc or nickel is difficult to remove from the solution, and if not removed interferes with the final color reaction. Folin uses silver lactate in lactic acid as a precipitant for uric acid, but the difficulty with lactic acid lies in the fact that it gives a blue color with Folin's phenol reagent; a fact which was overlooked by Folin and may in part be responsible for his high figures. Nevertheless, this method of precipitating the uric acid was retained, but the error caused thereby is now corrected by addition of a corresponding amount of lactic acid to the standard. Benedict's innovation of stabilizing the color by addition of sodium sulfite is omitted. Due to the more complete precipitation of the proteins and the higher dilution in which the readings are made, the dirty green tinge which sometimes occurs with Benedict's method does not appear. Finally another change has been made in the method: due to the higher dilution no addition of water to make up to 50 or 100 cc. as in Folin's or Benedict's methods is necessary, and there is substituted a manipulation of the final filtrate in graduated test-tubes which greatly increases the accuracy of the method and makes possible the detection of conjugated phenols in very minute quantities.

TABLE I.

*Recovery of Phenols Added to Blood.**

Before addition of phenols.	After addition of 5.81 mg. *p*-cresol† to 100 cc. of blood.
mg. per 1,000 cc.	*mg. per 1,000 cc.*
24.6	74.0
29.1	78.9
25.6	76.0
	Addition of 5 mg. phenol.
25.2	75.0

* The blood proteins must be precipitated immediately after the addition, as prolonged standing destroys a part of the phenols.

† 5.81 mg. of *p*-cresol are equal in color production to 5 mg. of phenol. The colorimeter reading is expressed as phenol.

TABLE II.

Accuracy of Separate Simultaneous Determinations of Phenol.

Determination.	Dog 1.	Dog 2.	Dog 3.
First	28.4	36.25	31.0
Second	28.75	37.1	30.6
Third	28.0	37.50	

Method.

The entire method is very simple and rapid. 10 cc. of blood are added to 50 cc. of distilled water in a 100 cc. Erlenmeyer flask. Then 10 cc. of 10 per cent sodium tungstate and 10 cc. of $\frac{2}{3}$ N sulfuric acid are added, the flask is closed with the thumb or rubber stopper, and vigorously shaken for a few seconds. To precipitate the proteins completely 10 cc. of aluminum cream are added and the flask is again shaken. The contents are transferred to a 100 cc. centrifuge tube and centrifugalized for 45 minutes. The supernatant fluid is filtered to the 45 cc. mark in a narrow 50 cc. graduate, 5 cc. of a 5 per cent solution of silver lactate in 5 per cent lactic acid are added, and the graduate is well shaken for 1 minute. After centrifugalization and filtration, the filtrate is ready to be examined for phenols. This last step is carried out as follows: only two narrow test-tubes are required —one graduated at 15 cc. and the other at 10 cc.—in which both the total and the free phenols are determined. Thus, any error due to the graduation of two sets of test-tubes is avoided. The procedure for the determination of *free* phenols is this: the 15 cc. tube is filled to the mark with the filtrate, 1 cc. of the phenol reagent[1] is added, and the tube is shaken. The excess silver precipitates out, the solution is filtered to the mark into the 10 cc. tube, and 5 cc. of 20 per cent sodium carbonate are added. This solution is now transferred to another test-tube in which the color develops to its maximum in about 20 minutes. The two graduated test-tubes are meanwhile used for the determination of *total* phenols. The 15 cc. tube is again filled to the mark with the same filtrate, 5 drops of concentrated HCl are added, and the tube is placed in a water bath at 100°C. for 10 minutes. Boiling of the contents of the tube is avoided and no loss of volatile phenols occurs, as was shown by repeating the determination with known amounts of phenol. If the tube has a diameter of 14 to 15 mm.,

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[1] Bell's modification of the reagent (Folin, O., and Denis, W., *J. Biol. Chem.*, 1916, xxvi, 508) is used, since the HCl which it contains is needed for the precipitation of the excess silver. It contains: 100 gm. of sodium tungstate, 20 gm. of phosphomolybdic acid, 50 cc. of phosphoric acid 85 per cent, 100 cc. of concentrated HCl. This is gently refluxed for 2 hours with 750 cc. of water, and at the end of the period of heating made up to 1,000 cc.

TABLE III.

Removal of Uric Acid by Precipitation.

Before addition of uric acid.	After addition of 5 mg. of uric acid to 100 cc. of blood.
Total phenols per 1,000 cc.	Total phenols per 1,000 cc.
mg.	*mg.*
23.5	23.8
25.5	25.5
29.0	29.5

TABLE IV.

Influence of Amino-Acids Normally Present in the Blood.

Before addition of casein digest.	After addition of casein digest (20 mg. of amino N to 100 cc.)
Total phenols per 1,000 cc.	Total phenols per 1,000 cc.
mg.	*mg.*
34.2	36.8
31.4	35.0
24.6	27.5

TABLE V.

Demonstration of Conjugated Phenols in Human and Dog's Blood by the Author's Method.*

Blood.	Total.	Free.	Conjugated.	
	mg. per 1,000 cc.	*mg. per 1,000 cc.*	*mg. per 1,000 cc.*	*per cent*
Human.				
Decompensated heart.....	31.2	28.5	2.7	8.6
Fracture of arm..........	37.2	33.2	4.0	10.7
Normal..................	42.8	38.0	4.8	11.2
Dog.				
Normal..................	29.4	28.2	1.2	4.1
	32.5	30.1	2.4	7.4
	38.1	35.2	2.9	7.6
	34.0	32.5	1.5	4.4
	27.4	24.4	3.0	10.9

* This table is not intended to give average values for human blood (for which purpose a much greater number of determinations must be made) but merely to demonstrate the presence of conjugated phenols.

the volume of the contents on cooling at the end of exactly 10 minutes is back to the graduation mark, so that no adjustment of volume is necessary. Then 1 cc. of the phenol reagent is added and the solution is treated in the same way as in the determination of free phenols. The difference between the total phenols and the free phenols represents conjugated phenols.

The standard is prepared as follows: 5 cc. of the stock solution of resorcinol (Benedict and Theis, 1), containing 5.81 mg. are placed in a 100 cc. volumetric flask, 0.5 cc. of concentrated HCl and 10 cc. of the silver lactate-lactic acid solution added, centrifugalized or filtered, and the filtrate is manipulated in the graduated test-tubes in the same manner as the blood filtrate in the determination of free phenols.

Recently there have been published several criticisms of Folin's phenol reagent (6, 7, 9), from which it appears that this reagent gives a blue color with a great many substances, and that it is by no means specific for phenols and the closely allied hydroxy-acids. It must be said, however, that most of these substances are normally not present in the blood, and that the normal constituents of the blood which interfere with the reaction can either be readily removed (uric acid, Table III), or are in such small concentration (amino-acids, Table IV), that their presence accounts, at best, for only a negligible fraction of the color developed. Further, in experiments such as reported in the later papers of this series, where we are dealing with the measurement of injected or ingested phenols, the color-producing effect of these interfering substances is entirely eliminated by a preliminary determination.

SUMMARY.

1. A method is described for the determination of phenolic substances in blood, which is based on Folin's method for the determination of phenolic substances in urine.

2. Contrary to the opinion of Benedict and Theis (1) conjugated phenols are present in human as well as in dogs' blood and can be demonstrated with the above method.

I am indebted to Professor W. R. Bloor and Dr. C. L. A. Schmidt of the Department of Biochemistry for their helpful advice and criticism in the development of this method.

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Reprinted from The Journal of Biological Chemistry
Vol. L, No. 2, February, 1922

THE PHYSIOLOGY OF THE PHENOLS.

II. ABSORPTION, CONJUGATION, AND EXCRETION.

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(Received for publication, December 12, 1921.)

With a suitable method at hand for the quantitative estimation of phenolic substances in the blood we took up a study and review of the factors concerned in the normal and abnormal metabolism of phenols in the body, directing particular attention to the liver. Many investigators have studied this question and have marshalled experiments to prove or disprove that the conjugation of phenols takes place in the liver, or again in the kidney, or elsewhere in the body. The best evidence to date is in favor of conjugation taking place in the liver, but in the face of contradictory experiments it must be admitted that much of this evidence is indirect and the question is at least open to debate. We may now review some of the recent work which concerns our thesis.

Baumann (1) noted a temporary accumulation of conjugated phenols in the liver of a dog poisoned with phenol, and showed that he could obtain nineteen times more phenol-sulfuric acid from that organ than from other organs or the blood. He suggested that the liver is the organ primarily concerned with the synthesis. Christiani and Baumann (4) tied off the ureters of a dog and showed that no accumulation of ethereal sulfates occurs in the blood. From this they concluded that, at least when the ureters are tied, the kidneys take no part in the conjugation of phenols. In other and more convincing experiments they ligatured all renal vessels and poisoned the dog with phenol. Synthesis occurred to the same extent as with normal dogs. From these experiments they concluded that if the kidneys are at all concerned with this reaction they are concerned with it to a negligible extent only. In the pursuit of the question of the participation of the kidneys in this synthesis Baumann and Herter (2) had already tried to perfuse the kidneys with blood containing phenol and sodium sulfate and had been unable to show any synthesis. Kochs (14) ground up liver, kidney, pancreas, and muscle, and added phenol plus sodium sulfate. He states that a moderate amount of conjugation was demonstrable in each case, and that negative results were obtained with thymus gland. Landi (15) repeated Kochs' experiments but could find no conjugation. From perfusion experiments with some of the organs he concludes that the intestine is the seat of the synthetic process. Lang (16) found small amounts of conjugated phenols in the urine of geese whose livers he had extirpated, and thinks that the liver is not the exclusive, although the most important, organ of conjugation. Herter and Wakeman (11) added equal amounts of phenol to blood and ground brain, muscle, kidney, and liver, and showed an increasing disappearance of phenols in the order given. They do not believe, however, that this disappearance is due to a conjugation with sulfuric acid; rather they favor a chemical destruction or a "loose combination of the phenol molecules with the molecules of the substance." Salta (19) thought that he could determine the place of conjugation by an analysis of various organs for phenol-sulfuric acid. The largest amount was found in the liver. Then follow in decreasing amounts in the order given: muscles, lungs, intestine, stomach, nerves. No conjugated phenols were found in the brain. From this he reasons that all these organs play a part in the synthesis. Embden and Glaessner (6), in a number of carefully controlled perfusion experiments, show that the conjugation takes place almost exclusively in the liver, although very small amounts of ethereal sulfates were found in the lungs and kidneys.

The work of Herter and Wakeman (11) is sometimes cited (5) as proof that other organs than the liver, *i.e.*, the intestinal epithelium, kidney, muscle, brain, blood, etc., have the ability, although to a lesser extent, to conjugate phenols with sulfuric and glucuronic acids. This statement, which is not in accordance with observable facts, is based on a misapprehension of the work of the first named authors. Although their experiments showed a disappearance of phenols when known amounts were added to and left in contact for some time with ground liver, muscle, kidney, etc., there is no evidence that this disappearance is due to a synthesis of phenol-sulfuric or glucuronic acids. In fact, Herter and Wakeman themselves state[1] that "the synthesis of indoxyl potassium sulphate cannot be accomplished by extirpated cells. It also seems improbable that the dead cells convert phenol into phenol-sulphuric acid." They then attempt to show[2] that this destruction of phenols is not in the nature of an oxidative process since they were unable to recover such oxidation products of phenol as hydroquinone or pyrocatechol. By subjecting liver pulp to various ferment-destroying agents (alcohol, boiling water, hot air, bichloride of mercury) and finding no decrease in the behavior towards phenol, these authors consider it highly improbable that ferments play any important rôle in this disposal of phenols, and they finally speculate[3] as to the probability of some "loose combination of the phenol molecules with the molecules of the cell substance."

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[1] Herter and Wakeman (11), p. 317.

[2] Herter and Wakeman (11), pp. 317–318.

It should be strongly emphasized that there is little experimental evidence for the statement that other organs than the liver have this ability to conjugate phenols. If the transformation of phenols by dead tissues is due to conjugation, it should be possible to hydrolyze these conjugated phenols by the addition of a few drops of HCl and the application of heat, and so recover the total amount of added phenol. That this is not the case is shown in Table VI in which are recorded the results of an experiment similar to those of Herter and Wakeman. Instead of using Millon's reagent the method described in the first paper of this series was used.

TABLE VI.

Blood and Liver Incubated with Phenols.

Blood blank.	Blood + 5 mg. phenol per 100 cc. and 2 hrs. incubation.	Same + 5 mg. $K_2S_2O_8$ per 100 cc.	Same hydrolyzed with 5 drops HCl and heat.
mg. per 100 cc.	*mg. per 100 cc.*	*mg. per 100 cc.*	*mg. per 100 cc.*
2.8	7.6	7.4	7.45
3.2	7.9	8.2	8.1
Liver as above.			
6.6	9.8	10.0	9.4
7.25	10.5	10.1	10.6

Known amounts of ingested phenols cannot be completely recovered in the urine or feces, and this loss is probably due to a similar reaction by which organ pulp disposes of phenol, *i.e.*, chemical oxidation, ferment action, or Herter and Wakeman's "loose combination."

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[3] Herter and Wakeman (11), pp. 319–320.

Tauber (22) fed phenol to a dog and found that as the dose was decreased. the amount of phenol so "oxidized" was increased. On feeding 240 mg. of phenol in water *per os*, he found that 53 per cent of the ingested phenol was oxidized in the body through oxalic acid to carbon dioxide. Dubin (5) found that 68.7 per cent of ingested phenol and 50.6 per cent of *p*-cresol could be recovered from the urine. When tyrosine was given, only 17.7 per cent of the amount of phenol corresponding to the amount of tyrosine administered was recovered. Friedländer (9) recovered only 25 per cent of ingested cresol from the urine of his dogs. Numerous other workers have noted the fact that ingested phenols cannot be quantitatively recovered from the excretions. Siegfried and Zimmermann (20) fed *p*-cresol to a dog and recovered both *p*-cresol and phenol, the sum of both amounting to 32 to 48 per cent of the *p*-cresol fed. Jonescu (13) administered cresols to dogs and noted that they were oxidized in the order of ascending toxicity. The percentage oxidation was as follows: *m*-cresol 50 to 53 per cent, *o*-cresol 65 to 69 per cent, and *p*-cresol 73 to 76.5 per cent. As an example of the powerful oxidation to which phenols may be subjected in the organism, Jaffé (12) has shown that the ingestion of benzene increases the urinary output of muconic acid, and suggests that this is due to the cleavage of the benzene ring into a straight chain compound. The whole subject of oxidases and phenolases is exhaustively treated in Oppenheimer's textbook (18) and need here not be further considered. ("Die Wirkung dieser Phenolasen ist neben der Oxidation, die niemals tiefgreifend ist, häufig eine Kondensation mehrerer Moleküle. Das hängt mit ihrem physiologischen Zwecke der Schaffung unlöslicher Stoffe zusammen.")

This oxidizing action on the phenols which has been noted by Tauber to increase as the amount of volatile phenol present decreased, would explain why normally there are such small amounts of conjugated phenols in the blood: the volatile phenols produced in the intestinal tract are probably to a large extent rapidly oxidized by the intestinal mucosa and the liver—and those which escape this process are *completely* conjugated by the liver. When the oxidizing mechanism is overwhelmed by large amounts of phenol, as occurs when phenol is ingested, more of that substance reaches the hepatic tissues unchanged and the relative amount of conjugated phenols is greater. Ordinarily this oxidizing mechanism is sufficient and much larger amounts of volatile phenols than are normally produced in the intestine are oxidized within a few minutes. This serves to strengthen the argument that free volatile phenols as such cannot remain in the blood stream for any considerable period.

It now appears probable that in its effort to dispose of phenol which reaches the circulation from the intestinal tract, the body makes use of two methods: one a process of oxidation which takes place to a considerable extent in the epithelial lining of the intestinal tract and in the liver and to a lesser extent in the other organs; the other a process of conjugation with sulfuric and glucuronic acids which takes place, as we shall later show, exclusively in the liver.

As regards the nature of the phenolic substances in the blood, there is no reason to suppose that they are essentially different from those in the urine. The most important literature dealing with that subject has been summarized by Folin and Denis (8), Dubin (5), and Tisdall (23).

EXPERIMENTAL OBSERVATIONS.

It seemed necessary to control diet factors in these experiments because of the observations of Underhill and Simpson (24) to the effect that urinary phenols vary directly with the protein intake, and with an increase in output of total phenols the percentage of free phenols remains constant. Some similar observations are recorded in Table VII which gives controls for the various diets under laboratory conditions.

Dogs weighing 20 to 35 pounds were used in these experiments. Mixed diet indicates a liberal amount of mixture of table scraps including cooked meat, macaroni, potatoes, bread, and bones.

The most important volatile phenols present in the body are *p*-cresol and phenol (7) and we, therefore, limited out experiments to these two substances focussing our attention upon *p*-cresol, as we found that complete conjugation occurs with it more rapidly than with any other phenolic substance investigated. At first we injected known amounts of phenol in a watery solution into the jugular vein of the animal and analyzed for total and free phenols the blood drawn just prior to and at stated intervals after the injection. Charts A and B give typical results of some of these early experiments. These experiments show that 5 minutes after the injection of 500 mg. of *p*-cresol or phenol the "theoretical concentration" of about 500 mg. per 1,000 cc. of blood has fallen to 80 mg. per 1,000 cc. We say "theoretical" advisedly, for the disappearance of phenols from the blood stream is so rapid that, due to the time lost during the slow injection this concentration is never approximated. The dog weighed 24 pounds and we may assume an approximate blood volume of 1,000 cc. This almost instantaneous disappearance of injected phenols is, we believe, due to two factors; a rapid and relatively uniform distribution throughout all tissues of the body, and oxidative processes also occurring throughout the body although not with equal rapidity in all tissues. The amount of *conjugated phenols* in the blood 5 minutes after the injection is only slightly greater than before injection, but it increases rapidly to a maximum about 1 hour after injection. At the end of the hour the *free phenols* have reached their pre-injection level and the conjugated phenols fall slowly (6 to 12 hours) back to their normal level.

TABLE VII.

Diet Factors and Blood Phenols.

Dog.	Total phenols per 1,000 cc. of blood.	Conjugated phenols.	Total phenols per 1,000 cc. of blood.	Conjugated phenols.
	Exclusive carbohydrate diet.		High protein diet.	
	mg.	*per cent*	*mg.*	*per cent*
21-56	26.5	7.0	39.4	8.0
21-95	23.4	5.5	42.2	7.0
21-95	24.0	6.4	45.9	8.4
21-98	31.7	7.4	38.8	6.6
21-80	25.0	8.0	43.0	7.8
	5 to 8 days fasting.		Mixed diet.	
21-98	28.1	9.0	36.8	4.0
21-105	23.5	6.3	37.9	6.1
21-80	29.0	7.0	28.2	11.0
21-80	32.4	7.4	35.0	5.8

As these curves are typical of a large number of determinations made on different dogs, it can be safely concluded from their examination that *p*-cresol is the more quickly oxidized or conjugated substance, for the fall of the free phenols back to the original level occurs sooner in every experiment.

The injection of such large amounts of highly poisonous substances invariably produces severe systemic reactions. Immediately after injection the pupils are widely dilated, the animal has convulsions and is unable to stand. In most cases the animal becomes clinically normal within 5 minutes. In some cases animals which have previously reacted moderately to repeated injections will not survive the unit dose. These intravenous injections are always dangerous and may destroy a valuable standardized animal. Because of this difficulty we examined the

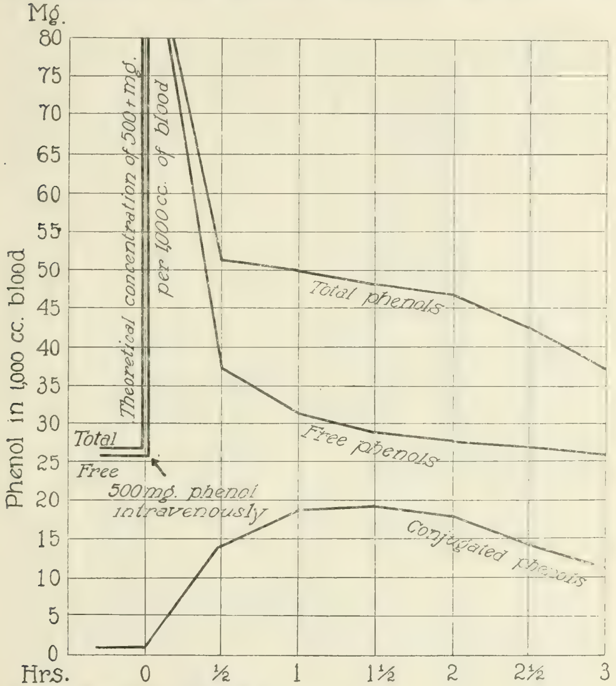

Chart A.

reaction to phenol solutions administered by stomach tube, and found that doses lethal for intravenous injection often produced no symptoms when given by stomach. An examination of Chart C shows that absorption from the intestine is sufficiently rapid and regular to be substituted in our experiments for intravenous injection. In fact, the ingestion of phenols is superior to the injection as it will be seen that complete conjugation of ingested phenols is more conspicuous. This, no doubt, is due to the fact that all the ingested phenols must pass through the liver before

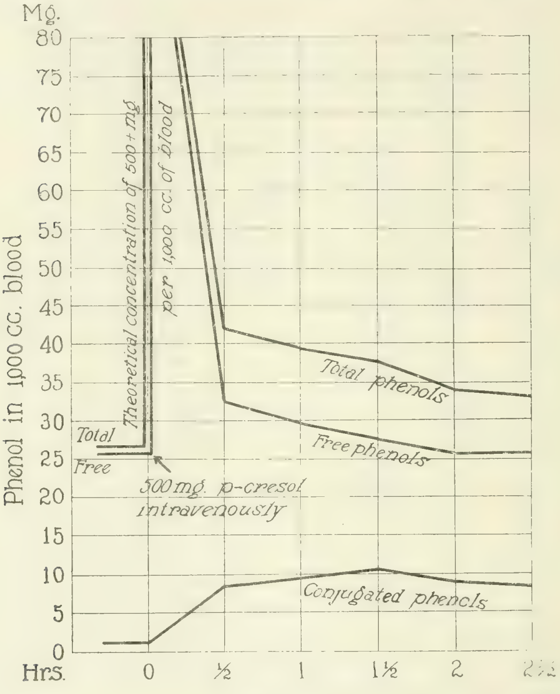

Chart B.

reaching the general circulation, while injected phenols are first distributed to all the tissues of the body and reach the liver much more slowly. The slight irregularities which sometimes occur in the ascending limb of the curve may be due to differences in stomach and intestinal absorption. In this connection it is interesting to recall the statement of Sollmann and Hanzlik (21) that the absorption of phenols is not a steady process, because of a slowing of the local intestinal circulation by these substances. A standard dose of 50 mg. in 10 cc. of water per pound body weight given by stomach tube has been used in all experiments reported in this paper except where otherwise stated.

A closer examination of the curves obtained by plotting the results of consecutive blood analyses reveals the following: immediately after *ingestion* both the total and free phenols begin to rise—the free somewhat slower than the total. The total phenols reach their greatest concentration between ½ and 1 hour after ingestion and after that time decline very slowly (6 to 12 hours) to their former level. The free phenols, after a slight rise within the first 20 or 30 minutes, quickly return to their old level (½ to 1 hour). In many cases the free phenols fall somewhat below that level. The resulting differences between the total and free phenols represent conjugated phenols which are plotted at the bottom of the charts. It will be seen that the conjugated phenols reach their greatest concentration 1 hour after ingestion and at this time the conjugation of ingested phenols is *complete;* an observation which corroborates Baumann (3), who showed that 1 hour after the intravenous injection of phenols the free phenols dis-

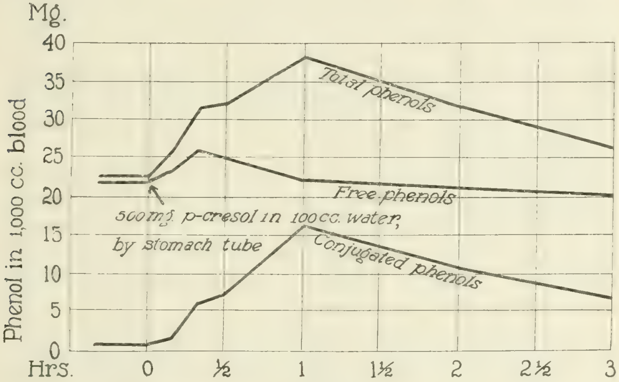

Chart C.

appeared from the system. After reaching the greatest concentration within 1 hour, the conjugated phenols are slowly excreted, corresponding to the slow fall of the total phenols.

From the fact that in a healthy animal the conjugation of very large amounts of ingested phenols is complete within 1 or 2 hours, it would appear unlikely that *free volatile phenols* are normally present in the blood. The conjugation of the small amount of volatile phenols produced in the intestine must be very rapid within the liver, and it is improbable that any free volatile phenols reach the general circulation under normal physiological conditions.

The bulk of the blue color (90 per cent and more) which is produced by Folin's reagent with the blood filtrate is due to substances other than true phenols. It may be due, among others, to hydroxy-acids and unidentified protein decomposition products as well as to carbohydrates and related substances (10, 17); the remainder of 10 per cent or less is produced by the conjugated phenols.

These three tables (Tables VIII, IX, and X) supplement each other and bring out several interesting points. The various organs compared with blood (Table VIII) contain substances which react with the phenol reagent and give larger figures for their "phenol" content. We have no reason to suppose that these reacting substances are true phenols.

The injection of phenol (Table IX) gives an immediate increase in phenol content of the blood and tissues. There is probably a pretty uniform distribution of the phenols in the blood and tissues but we record in analyses about 40 mg. increase in blood and 25 to 30 mg. increase in the parenchymatous organs.

After an hour's interval following a phenol injection (Table X) we note a uniform distribution of conjugated phenols in the blood and organs.

From these facts we wish to assume that free phenols, when injected into the blood stream, are distributed throughout the living tissues. After conjugation by the liver they again diffuse out to the tissues before being excreted (Table X). When phenols are given by stomach no such distribution of *free phenols* occurs because they are conjugated by the liver before reaching the general circulation. This last fact explains why doses of phenol, lethal when injected into the blood stream, show no effects when given by stomach tube, but are lethal once again when administered by stomach tube to a dog whose liver has been seriously injured.

TABLE VIII.

Distemper Dog, Killed with Chloroform.

Organ.	Total phenols per 1,000 gm.	Free phenols per 1,000 gm.	Conjugated phenols.
	mg.	*mg.*	*per cent*
Blood	22.3	20.8	1.5
Kidneys	65.8	65.8	0.0
Liver	71.3	70.1	1.2
Spleen	59.4	57.9	1.5

TABLE IX.

Dog Killed by Injection of Phenol into Jugular Vein, Death 5 Minutes after Injection.

Organ.	Total phenols per 1,000 gm.	Free phenols per 1,000 gm.	Conjugated phenols.
	mg.	*mg.*	*per cent*
Blood	60.0	56.6	3.4
Kidneys	92.4	91.2	1.2
Liver	96.8	90.1	6.7
Spleen	93.2	92.2	1.0
Muscle	88.4	88.4	0.0

TABLE X.

Dog Killed by Chloroform 1 Hour after Injection of Phenol.

Organ.	Total phenols per 1,000 gm.	Free phenols per 1,000 gm.	Conjugated phenols.
	mg.	*mg.*	*per cent*
Blood	50.0	33.3	16.7
Kidneys	117.6	100.0	17.6
Liver	117.6	100.0	17.6
Spleen	108.6	92.4	16.2

Baumann found nineteen times more conjugated phenol in the liver than in the blood of a dog poisoned with phenol. One of our dogs succumbed to an overdose of phenol given by stomach and we were able to confirm Baumann's observation. It is probable that in cases of fatal phenol poisoning by stomach the phenol absorbed from the intestinal tract is temporarily held by the liver and that there is not sufficient time nor proper circulation before the death of the animal for complete distribution to the tissues. Table IX shows that no such concentration of phenols in the liver occurs when the fatal poisoning is caused by injection into the jugular vein.

DISCUSSION.

We may review our conception of phenol metabolism somewhat as follows:

A small part of the tyrosine of the food proteins is broken down by bacterial action into hydroxy-acids such as *p*-oxyphenylproprionic, *p*-oxyphenylacetic, and *p*-oxybenzoic acids, and into volatile phenols, primarily *p*-cresol and phenol. The hydroxy-acids which have no toxic effects are not subjected in any noticeable degree to oxidation or conjugation and are practically completely excreted in the urine in the free state. The volatile phenols which are very toxic even in small amounts are dealt with in an entirely different way. More than half of these toxic phenols are oxidized by the intestinal mucosa, body fluids, and liver parenchyma. The remainder is conjugated in the liver with sulfuric or glucuronic acids. After passing from the liver the conjugated phenols are uniformly distributed to all the tissues and are rapidly eliminated by normal kidneys probably within 12 hours. From the experiments given in the following paper we feel confident that the synthesis of phenol-sulfuric and phenol-glucuronic acids takes place only in the hepatic parenchyma.

SUMMARY.

Intravenous injection of phenols is followed by a prompt and uniform distribution of such substances in the body fluids and tissues.

Following such injection we note a rapid disappearance of free phenols from the blood and a rapid increase in conjugated phenols. This rise in conjugated phenols usually reaches a maximum within 1 hour and thereafter slowly declines with excretion. We note too a uniform distribution of these conjugated phenols throughout the body fluids and tissues.

Ingestion of phenols gives a totally different picture. With a sufficiently large dose some free phenols appear in the blood for a short period—rarely more than 30 minutes. The conjugated phenols show a maximum rise during the first and second hours and subsequent decrease due to renal elimination.

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Reprinted from The Journal of Biological Chemistry
Vol. L, No. 2, February, 1922

STUDIES OF LIVER FUNCTION.

III. PHENOL CONJUGATION AS INFLUENCED BY LIVER INJURY AND INSUFFICIENCY.

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(Received for publication, December 12, 1921.)

In a recent article dealing with the study of liver function Delprat and Whipple (2) review some of the difficulties arising in this work. We refer to that paper for a discussion of the many factors which complicate a critical study of hepatic function, liver injury and repair, together with the great reserve capacity of the liver cells. The ideal liver function test is not at hand and any studies in this difficult field should be of interest alike to the physiologist and the physician. We must keep in mind that any satisfactory *liver function test must include some factor of strain or load* to determine the upper limits as well as the lower levels of liver function.

EXPERIMENTAL OBSERVATIONS.

Tables XI to XVI give the results of some of the experiments we have carried out. The general plan of these experiments was as follows: the dog was first standardized, either after several days of fasting or several days of carbohydrate diet in order to determine the time required by the liver to conjugate the standard dose of *p*-cresol. Liver injury was then produced by one of a number of methods and another test was made to observe the capacity of the injured liver. Tables XI and XIV are given as representative of over a score of standardization experiments on carbohydrate diet or fasting. All of them show surprising uniformity. The percentage conjugation of added phenols and the time required to do this work give a fairly accurate idea of the ability of the liver to conjugate phenols. In the calculation of the *percentage conjugation* of *added phenols* the assumption is made that the free and total phenols present before ingestion remain constant during the experiment and are not influenced by any of the procedures such as the repeated bleeding or the ingestion of fluid, etc. That this is actually the case is shown in Table XIV where one of three control experiments is reported. These experiments were similar in every way to the other tests performed, except that, instead of the phenol solution, a corresponding amount of water was given by stomach tube.

It may be advisable to explain by example how the *percentage conjugation* of added phenols is calculated:

	Total phenols.	Free phenols.
	mg.	*mg.*
Before ingestion	24	22
After "	28	24

The increase of total phenols after ingestion over total phenols before ingestion is 4 mg. The increase of free phenols after ingestion over free phenols before ingestion is 2 mg. The difference between the two, 2 mg. (or 50 per cent) of the added phenols, has been conjugated. The percentage conjugation of added phenols at any given time after ingestion may, therefore, be calculated by the following simple algebraic formula.

$$\frac{a-b}{c-d}.100 = \text{percentage conjugation}$$

where a represents free phenols after ingestion, b free phenols before ingestion, c total phenols after ingestion, d total phenols before ingestion. In the case above

$$\frac{24-22}{28-24}.100 = 50 \text{ per cent}$$

We have used the method of Davis and Whipple (1) to produce standard liver injury and necrosis with consequent impairment of function. All operative procedures were done under surgical ether anesthesia.

TABLE XI.

Chloroform Injury and Eck Fistula Experiments.

Dog 21-100. White bull, female.

Time.	Total phenols per 1,000 cc.	Free phenols per 1,000 cc.	Conjugated phenols in percentage of total.	Conjugation of added phenols.
Mar. 25. Standardization on carbohydrate diet.				
	mg.	*mg.*	*per cent*	*per cent*
Before ingestion..............	23.1	22.2	3.9	
After "				
10 minutes....................	26.7	24.0	10.0	50.0
20 "	29.4	23.8	19.0	75.0
30 "	29.4	22.5	27.0	95.0
1 hour........................	33.3	22.2	33.3	100.0
2 hours.......................	30.0	21.1	29.7	
Apr. 16. Chloroform anesthesia (1¼ hrs.). Apr. 18, *p*-cresol ingestion.				
Before ingestion..............	49.3	48.1	2.4	
After "				
10 minutes....................	53.5	52.1	2.6	4.8
20 "	54.9	50.5	8.0	57.0
30 "	55.6	50.5	9.2	62.0
1 hour........................	54.1	47.6	12.0	100.0
2 hours.......................	59.4	54.1	8.9	
Apr. 21. Eck fistula operation. Apr. 27, *p*-cresol ingestion.				
Before ingestion..............	21.1	20.7	1.4	
After "				
10 minutes....................	25.0	24.4	2.4	5.1
20 "	28.4	26.7	6.0	17.8
30 "	30.0	27.0	10.0	29.2
1 hour........................	30.7	26.7	13.2	37.4
2½ hours......................	30.0	24.7	17.7	55.0
5½ "	25.1	22.4	10.8	57.5
May 13. *p*-Cresol ingestion, conjugation much decreased.				
Before ingestion..............	35.0	34.0	2.8	
After "				
10 minutes....................	37.8	36.2	4.2	21.4
20 "	39.4	37.0	6.1	31.8
30 "	39.4	35.7	9.4	61.3
1 hour........................	42.0	40.0	5.0	14.3
2 hours.......................	41.0	39.0	4.9	16.6

TABLE XI—*Concluded.*

Time.	Total phenols per 1,000 cc.	Free phenols per 1,000 cc.	Conjugated phenols in percentage of total.	Conjugation of added phenols.
May 20. Ligation of hepatic artery. May 24, *p*-cresol ingestion.				
	mg.	*mg.*	*per cent*	*per cent*
Before ingestion	27.0	25.0	7.4	
After "				
15 minutes	35.7	33.7	5.6	0.0
30 "	41.5	39.0	6.0	3.4
1 hour	45.4	42.6	6.2	4.3
2 hours	47.6	45.2	5.0	2.0
4½ "	40.8	36.7	10.0	15.2
May 25. Death. Hepatic insufficiency				

Experimental Protocol of Dog 21–100 (See Table XI).

Mar. 25, 33 lbs., healthy. Standardized with 1,650 mg. of *p*-cresol after 4 days carbohydrate diet. No reaction. Apr. 16, 75 minutes chloroform after 4 days fasting. Apr. 18, 35 lbs., 1,750 mg. of *p*-cresol, no reaction. Conjugation about one-half normal. Apr. 21, Eck fistula operation. External surface of liver shows that the chloroform injury has not been completely repaired—the centers of the lobules are hyperemic and stand out distinctly from the opaque periphery. Apr. 27, 30 lbs., 1,500 mg. of *p*-cresol. ½ hour after ingestion the dog is severely intoxicated. Conjugation about one-third normal. Operative wound is partly open and infected. May 1, wound sewed up. May 3, wound again open and sewed up. May 13, slight muscle tremors—prominent distension of abdominal cutaneous veins. 32 lbs., 1,600 mg. of *p*-cresol. No reaction, but very weak. Conjugation about one-third normal. May 20, no tremors, no ascites, collateral circulation less prominent than on May 13. 30 lbs. Arch of hepatic artery tied in two places at 11 a.m. 1,500 mg. of *p*-cresol by stomach tube immediately after operation. Very severe reaction which may, in part, have been due to the ether. Temperature at 1 p.m. 36.2°; at 2 p.m. 37.2°; at 3 p.m. 37.9°. Conjugation one-fourth normal. May 24, very weak and slight muscle tremors. Hematocrit red cells 38 plus. 25 lbs. 1,250 mg. of *p*-cresol, severe reaction. Conjugation less than one-fourth normal. May 25, died 5 days after ligation of hepatic artery.

Autopsy.—May 25. Thorax, heart, and lungs normal. Spleen fibrous and rather pale. Serous surfaces clean except plastic adhesions and yellowish fibrinous exudate about site of hepatic artery operation. Stomach and intestines not abnormal. Kidney and pancreas negative. Hepatic artery ligated in two places and completely occluded.

Eck fistula about 6 mm. long and clean. There must have been considerable flow through this opening or it would have closed. The ligature above the Eck fistula on the portal vein did not completely occlude the lumen. Lumen probably about 1 mm. in diameter. The knot must have slipped before the second tie was made.

Liver is atrophic but not exactly like the usual Eck fistula specimen. It is not as translucent. The lobules are small and brown at the margins—yellow in the centers, probably necrotic. Some areas are swollen, yellow, and opaque. The lobules here are larger and yellow—necrosis probable. These areas are not numerous, but pretty sizable, 2 × 2 × 5 or 6 cm. for the largest—the volume is estimated as about one-tenth or less of the liver parenchyma.

Microscopic Examination.—Spleen, much pigment and phagocytes. Liver, some sections show extensive hyaline necrosis, involving the centers of the lobules up to 90 per cent in extent—few liver cells escape at the margins of the lobules. Other sections show central atrophy alone (usual Eck picture) with phagocytes full of lipochrome pigment. Others show some evidence of repair, enlarged liver cells, mitoses, etc. Other organs of no importance.

Most instructive are experiments performed on two Eck fistula dogs.

Standardized on Mar. 25, Dog 21-100 showed 95 per cent conjugation within 30 minutes (Table XI). On Apr. 16 a liver injury of about 60 or 70 per cent was produced by chloroform. Table XI, experiment on Apr. 18, shows slightly less than 50 per cent reduction of function. On Apr. 21 the Eck fistula operation was performed. On Apr. 27 the test with *p*-cresol revealed a one-third normal conjugation. A repetition of this test on May 13 shows a slight improvement in function. The arch of the hepatic artery was ligated in two places on May 20 and on testing with *p*-cresol we found that there remained less than 3 per cent of the original capacity of the liver to conjugate phenols. Death of the dog was due to liver insufficiency.

The story of the other Eck fistula dog is similar.

Table XII shows that Dog 21-105 exhibited perfectly normal conjugation when standardized on a carbohydrate diet. On Apr. 5 we performed an Eck fistula operation and a conjugation test on Apr. 12. It appears that only 27.8 per cent of the added phenols had been conjugated 2 hours after ingestion, whereas before the operation 100 per cent conjugation occurred in 20 minutes—the function of the liver on Apr. 12 we may estimate as about 10 per cent of normal. On Apr. 20 another test was made and some improvement noted. The conjugation at this time amounted to about 25 per cent of normal. On May 16 we ligated the hepatic artery in one place, leaving some of the collaterals to the liver patent. It will be seen that the liver function after this operation amounted to somewhat more than 5 per cent of normal.

TABLE XII.

Eck Fistula Experiments.

Dog 21-105. Black, female.

Time.	Total phenols per 1,000 cc.	Free phenols per 1,000 cc.	Conjugated phenols in percentage of total.	Conjugation of added phenols.
Mar. 31. Standardization on carbohydrate diet.				
	mg.	*mg.*	*per cent*	*per cent*
Before ingestion	24.3	23.4	3.7	
After "				
10 minutes	26.0	23.5	9.6	94.0
20 "	28.2	22.0	22.0	100.0
30 "	29.0	22.5	22.4	100.0
1 hour	35.0	21.8	37.7	100.0
2 hours	33.0	23.0	30.0	100.0
Apr. 5. Eck fistula operation. Apr. 12, *p*-cresol ingestion.				
Before ingestion	25.5	24.7	3.1	
After "				
10 minutes	33.1	31.2	5.7	14.4
20 "	34.2	32.2	5.8	13.8
30 "	36.8	34.5	6.2	13.2
1 hour	37.7	34.5	8.5	19.6
2 hours	36.3	32.5	10.4	27.8
Apr. 20. 15 days after Eck fistula operation.				
Before ingestion	27.0	26.7	1.1	
After "				
10 minutes	38.0	36.2	4.7	13.6
20 "	38.0	35.1	7.6	23.6
30 "	42.5	38.5	9.4	23.9
1 hour	42.0	35.7	15.0	40.0
2 hours	37.0	30.0	18.9	67.0
3 "	33.5	25.0	25.3	100.0
May. 16. Immediately after partial ligation of hepatic artery. Conjugation greatly decreased.				
Before ingestion	26.7	26.0	2.6	
After "				
15 minutes	43.5	41.7	4.1	6.5
30 "	47.0	44.7	4.8	7.8
1 hour	52.5	48.1	8.3	14.3
1½ hours	52.0	46.9	9.8	17.3
2½ "	40.9	35.0	14.4	36.6
5 "	37.0	30.2	18.3	59.2

May 18. Death. Hepatic insufficiency and peritonitis.

Experimental Protocol of Dog 21–105 (See Table XII).

Mar. 27, carbohydrate diet begun. Mar. 31, 29 lbs., slight distemper. Standardized with 1,450 mg. of *p*-cresol. No reaction. Apr. 5, Eck fistula operation. No food. Apr. 6, water and bread. Apr. 7, regulation diet: carbohydrates, vegetables, kaolin, and bones. No meat or milk. Apr. 12, 26 lbs., slight distemper. Moderate ascites. 1,300 mg. of *p*-cresol by stomach tube. Moderately severe reaction. ½ hour after ingestion dog falls down occasionally when trying to walk. 1 hour after dog does not fall down but is still very shaky. Phenol conjugation is about one-tenth normal. Apr. 20, 30 lbs., no distemper. Severe ascites. Collateral circulation on abdominal wall is prominent. 1,500 mg. of *p*-cresol by stomach tube. Moderate reaction. A half hour after ingestion the dog sways from side to side when trying to walk but does not fall. Phenol conjugation about one-fifth normal (a little better than on Apr. 12). May 16, 26 lbs., moderate ascites. Ligature on hepatic artery in only one place, 1,300 mg. of *p*-cresol. Moderate reaction. Dog has apparently recovered late in afternoon. Phenol conjugation less than one-tenth normal. Hematocrit red cell 42 per cent. May 18, dog died 32 to 38 hours after last operation.

Autopsy.—May 18. Ascitic fluid less than at operation—now about 100 cc. The fluid is turbid and the serous surfaces are injected—there is a perforation in the first third of the duodenum and shortly before death there evidently had been an escape of intestinal contents with recent peritonitis. Thorax, heart, and lungs negative. Blood clots normal. Spleen small and fibrous; not much blood. Adhesions about the site of the first operation are numerous and bled easily at the second operation. Liver small, decidedly yellow, due to fat. Lobules show much injury (fat) and perhaps necrosis—there is no edema and the veins are clear. Fistula is about 5 mm. in length and is less than one-half its original length. The edges are smooth and there is no thrombosis. The passage of blood through it was obviously difficult and caused the portal stasis, development of collaterals, and ascites. Intestinal tract negative in general. Duodenum shows a sharp punched out area about 3 × 1 cm. due probably to ligature of the hepatic artery. Kidneys negative.

Microscopic Examination.—Much fat in liver cells (large and small droplets), central necrosis is abundant—about one-third of cell lobules. Kidneys negative. Death explained by liver injury plus terminal duodenal perforation.

Chloroform poisoning gives considerable information as to the conjugation of phenols in the liver. It also points out the fact that *this method is inadequate to measure the high limits of liver capacity*. For example, given a chloroform injury of approximately one-third of the liver parenchyma we may expect a normal or almost normal conjugation of phenols after administration of the unit dose with the usual routine technique. This means that our test does not reveal the maximum capacity of the normal liver in conjugation of phenols. When we have a chloroform liver injury of one-half to two-thirds of the liver lobule we can then demonstrate a considerable impairment of liver conjugation (compare Table XI, Experiment of April 18). With more advanced liver injury we note a great drop in liver conjugation (compare Table XIV, Experiment of April 22). Finally with a *lethal chloroform injury* involving the greater part (90 per cent or more) of each liver lobule (Table XIII, Dog 21–113) we observe *zero liver conjugation.*

Phosphorus injury gives a similar picture of impaired liver function (Table XIII). It is more difficult to estimate the amount of liver injury in phosphorus poisoning as cell necrosis is not a conspicuous feature of the injury. We note two experiments in Table XIII to show liver function impairment. The injury was fatal in one experiment and very severe in the second animal, yet there was a certain amount of phenol conjugation in both experiments.

We observed no impairment of liver function as shown by phenol conjugation in bile fistula dogs. It is interesting to recall that one liver function test (phenoltetrachlorphthalein) shows a distinct fall in output in bile fistula dogs indicating that the chronic cholangitis which is usually present in such animals interferes with the elimination of phenoltetrachlorphthalein but not with the conjugation of phenols.

Another important control is given by dogs sick with *distemper*, as it might be argued that intoxication of any sort might disturb this conjugation of phenols. One animal (Table XVI) was profoundly prostrated with acute distemper and died shortly after the completion of the experiment yet a normal conjugation followed the administration of the unit dose of *p*-cresol. Autopsy showed the familiar lesions of acute distemper and nothing else. Under these conditions therefore the capacity of the liver to conjugate phenols is in nowise impaired.

From an examination of Table XI, Experiment of April 18, it will be seen that following chloroform injury the initial concentration of total phenolic substances in the blood is more than twice, and, in some cases (fatal chloroform injury, Table XIII),

TABLE XIII.

Chloroform and Phosphorus Injury of Liver.

Dog 21-113. Mongrel, female.

Time.	Total phenols per 1,000 cc.	Free phenols per 1,000 cc.	Conjugated phenols in percentage of total.	Conjugation of added phenols.
May 2. Chloroform anesthesia, 1½ hours. Liver necrosis almost total. Conjugation zero.				
May 3. *p*-Cresol by ingestion.				
	mg.	*mg.*	*per cent*	*per cent*
Before ingestion	103.0	99.2	3.0	
After "				
10 minutes	111.0	107.0	3.6	2.5
20 "	121.5	117.0	3.7	3.8
30 "	124.0	121.0	2.4	0.0
1 hour	135.0	131.0	3.0	0.6
2 hours	133.0	130.0	2.3	0.0
Dog died 2 days after injury. Microscopic examination shows only 1 to 2 rows of surviving liver cells about portal veins.				
Dog 21-112. Black, male. Phosphorus injury, fatal. Conjugation impaired.				
Before ingestion	42.5	42.0	1.2	
After "				
10 minutes	49.5	47.0	5.5	28.5
20 "	51.5	47.6	7.6	37.7
30 "	52.6	46.0	12.5	60.4
1 hour	60.5	50.0	17.3	55.5
2½ hours	58.5	38.5	34.2	100.0
2 mg. of phosphorus per pound body weight, in oil subcutaneously. Test made 1 day after injury. Dog died 36 hours after injection. Autopsy shows fatty degeneration of liver.				
Dog 21-76. Mongrel, male. Phosphorus injury, sublethal but severe. Conjugation impaired.				
Before ingestion	26.7	26.0	2.7	
After "				
10 minutes	32.6	27.0	17.1	83.0
20 "	35.6	29.2	17.7	61.3
30 "	40.8	31.5	22.7	61.0
1 hour	40.0	28.2	29.5	83.5
2 hours	34.2	25.2	26.3	100.0
2 mg. of phosphorus per pound body weight, in oil subcutaneously. Test made 2 days after injury. Dog recovered very slowly.				

four times as high as in the blood of fasting normal dogs or dogs on a carbohydrate diet. We have no definite knowledge regarding all the factors responsible for this pronounced increase, but it is interesting to speculate about some of them. It has been shown by Dubin (3) that in a fasting dog the concentration of urinary phenols, after an initial drop rises considerably. In our cases

TABLE XIV.

Chloroform Liver Injury and Controls.

Dog 21-80. Brown, female.

Time.	Total phenols per 1,000 cc.	Free phenols per 1,000 cc.	Conjugated phenols in percentage of total.	Conjugation of added phenols.
Apr. 15. Standardization after 4 days fasting.				
	mg.	*mg.*	*per cent*	*per cent*
Before ingestion	30.8	29.4	4.5	
After "				
10 minutes	35.2	30.2	14.2	98.0
20 "	37.8	30.2	20.1	89.0
30 "	38.5	28.0	27.3	100.0
1 hour	40.0	28.5	28.7	100.0
2 hours	36.4	28.2	22.5	100.0
Apr. 20. Chloroform anesthesia 1 hour, 20 minutes. Apr. 22, *p*-cresol by ingestion.				
Before ingestion	31.3	30.0	4.1	
After "				
10 minutes	43.5	41.0	5.7	9.8
20 "	48.5	43.6	10.1	20.9
30 "	47.8	41.8	12.5	28.4
1 hour	46.5	40.0	14.0	34.2
2 hours	40.0	33.0	17.5	65.2
Effect of repeated bleedings. Apr. 28. Water given instead of phenol solution.				
Before ingestion	27.2	26.8	1.4	
After "				
10 minutes	27.0	26.9	0.4	
20 "	26.8	26.8	0.0	
30 "	27.5	27.2	1.0	
1 hour	26.9	26.7	0.7	
2 hours	26.5	26.4	0.4	

TABLE XV.

Bile Fistula Control.

Bile fistula dog (old white bull, female).

Time.	Total phenols per 1,000 cc.	Free phenols per 1,000 cc.	Conjugated phenols in percentage of total.	Conjugation of added phenols.
	mg.	*mg.*	*per cent*	*per cent*
Before ingestion	22.7	21.5	5.3	
After "				
10 minutes	31.2	28.3	9.3	20
30 "	34.5	22.7	34.2	89
1 hour	31.1	22.7	27.0	86
2 hours	30.8	20.8	32.4	100

Bile fistula of over 2 years duration—dog normal.

TABLE XVI.

Acute Distemper Intoxication—Control.

Dog 21-98. Black-brown, long haired female.

Time.	Total phenols per 1,000 cc.	Free phenols per 1,000 cc.	Conjugated phenols in percentage of total.	Conjugation of added phenols.
Mar. 23. Standardization.				
	mg.	*mg.*	*per cent*	*per cent*
Before ingestion	28.3	27.8	1.7	
After "				
10 minutes	37.0	33.3	10.0	36.5
20 "	43.1	37.4	13.2	34.7
30 "	47.1	40.0	15.0	35.1
1 hour	41.7	28.9	30.7	91.7
2 hours	37.7	25.4	32.6	100.0
Apr. 13. Terminal distemper.				
Before ingestion	29.0	28 4	2.0	
After "				
10 minutes	38.0	33.0	13.1	48.9
20 "	42.4	37.0	12.7	36.0
30 "	45.2	38.3	15.2	38.9
1 hour	40.0	29.8	25.5	87.2

Very severe distemper, toxic condition, slight reaction to cresol after 10 minutes, from which dog completely recovered in 30 minutes. Died of distemper after 1 hour. Postmortem: No pneumonia.

of chloroform injury the period of fasting (3 to 4 days) was certainly not long enough to account for the tremendous increase; this is further controlled by the normal fasting dogs (Table XIV and others) in which no such increase occurs. It has been pointed out that the increase of urinary phenols in prolonged fasting is due to a great destruction of proteins. This cell destruction may be one of the factors producing the rise of blood phenols in liver injury. As mentioned before, proteins and their decomposition products such as tyrosine, tryptophane, and other easily oxidizable substances give the blue color with the phenol reagent, and since the increase of conjugated, volatile phenols in these cases is not proportional to the increase in total phenols (the conjugated phenols being practically the same as in normal dogs) the bulk of the additional color-producing bodies may be proteins and their decomposition products other than phenols, or sugars and related substances (Gortner and Holm, 4; Levine, 5).

It was shown in the preceding paper that a large part of the absorbed phenols is destroyed by a process of oxidation entirely different from the synthetic process by which conjugated phenols are produced. It is possible that this oxidative destruction which goes on in most tissues, but to greatest extent in the liver and in the intestinal epithelium, is inhibited or at least greatly reduced by the presence of injured liver tissue, thus adding to the increase in phenolic substances. This seems to be substantiated by the fact that in dogs injured by chloroform the absolute rise in blood phenols after ingestion of *p*-cresol is always greater than in normal dogs. Table XI, Experiment of April 18, and Table XIII, Dog 21–113 show further that with chloroform injury the excretion of ingested phenols may be less rapid than in normal dogs—another less important factor in the increased concentration of phenolic substances in the blood. Delprat and Whipple (2) showed that there is no impairment of *renal* function following a chloroform anesthesia as measured by the elimination of phenolsulfonephthalein.

In accounting for this increased concentration of "phenolic reacting substances" in blood in cases of chloroform injury, we have, in addition to whatever unknown factors may play a part, these possibilities: (1) an increased destruction of body proteins, particularly liver proteins, by chloroform; (2) an inhibition or lessening of the oxidative destruction of absorbed phenolic substances; and (3) a slowing of the excretion of phenolic substances.

It is interesting to note that this rise in blood "phenols" seldom occurs in dogs whose liver has been injured with phosphorus. Even fatal phosphorus injury shows only a slight increase over the normal level (Table XIII). Nor does this increase occur with bile fistula or Eck fistula dogs. This indicates again the presence of certain "phenol-reacting" substances in the blood of dogs poisoned with chloroform but not necessarily true phenols alone.

DISCUSSION.

The cause of death in Eck fistula dogs has long been a puzzle to physiologists. It has been claimed by many workers that this peculiar intoxication was due to the absorption of the toxic amino-acids as it is well known that heavy meat feeding will precipitate the characteristic intoxication. Unpublished experiments of Van Slyke and Whipple show that there is no abnormal heaping up of amino nitrogen in the blood during periods of meat feeding in Eck fistula dogs. No amino-acids, such as have been noted in severe cases of chloroform poisoning and fatal liver injury, appear in the urine of these Eck fistula dogs. The experiments given in this paper indicate clearly that the Eck fistula liver is incapable of normal conjugation of one toxic radicle, (*p*-cresol). This disability is noted during periods of normal health as indicated by clinically normal reactions. It is at least possible that this impairment of the conjugating powers of the liver is responsible for the toxic developments in the Eck fistula dog. We note that there is no heaping up in the blood of the Eck fistula of any phenol-reacting substances. It will be of considerable interest to study this reaction in the Eck fistula dogs on a high meat diet and during periods of the characteristic Eck fistula intoxication. It is to be recalled that these Eck fistula dogs were maintained on a diet of rice, bread, milk, and bones.

It is significant to note in the tables that severe poisoning with phosphorus will not cause a great rise in the total blood phenols but equally severe poisoning with chloroform will give very high figures for total blood phenols. One suspects that cell necrosis which is so conspicuous in chloroform poisoning is responsible for this difference. This reasoning suggests that a considerable part of the total phenol of the blood in chloroform poisoning may be due to "*phenol-reacting substances*" quite apart from the phenolic substances.

The method we have used is of interest to investigators but as yet of little practical value to internists. *p*-Cresol is too toxic to be used clinically but further study may enable us to suggest some non-toxic radicle which will test these synthetic activities of the liver parenchyma. It is of some significance that this method is a specific test of liver function, as we have evidence that other body cells are not concerned in the conjugation of phenols.

SUMMARY.

The conjugation of phenols in the body is in nowise disturbed by bleeding periods, by the presence of a bile fistula nor by a lethal intoxication (distemper). Under the conditions of these experiments the reaction to ingestion of a unit dose of *p*-cresol is uniform and associated with a constant amount of phenol conjugation which can be measured quantitatively.

The presence of an Eck fistula modifies this reaction and *reduces* the amount and speed of phenol conjugation. At times the Eck fistula liver function of conjugation may fall to one-third or even to one-tenth of the normal. When the liver circulation is further impaired by partial ligation of the hepatic artery in an Eck fistula dog, we may observe a fall in phenol conjugation to 3 or 5 per cent of normal. Liver exclusion, therefore, will eliminate phenol conjugation.

The presence of a slight liver injury due to chloroform or phosphorus may not modify the phenol conjugation. Extensive liver injury due to these poisons will always lessen phenol conjugation. Extreme and *fatal liver injury* (chloroform) *will reduce phenol conjugation to zero.*

These observations lead us to conclude that *phenol conjugation* is a function of *liver parenchyma cells* and of no other body cells.

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2. Delprat, C. D., and Whipple, G. H., *J. Biol. Chem.*, 1921, xlix, 229.
3. Dubin, H., *J. Biol. Chem.*, 1916, xxvi, 70.
4. Gortner, R. A., and Holm, G. E., *J. Am. Chem. Soc.*, 1920, xlii, 1678.
5. Levine, V. E., *Science*, 1920, lii, 612.

Pigment Metabolism and Regeneration of Hemoglobin in the Body

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PIGMENT METABOLISM AND REGENERATION OF HEMOGLOBIN IN THE BODY *

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The words "pigment metabolism" should mean the general exchange and balance in the body of all pigment substances—the income of pigment forming material, the story of pigment building in the body, the disposition of recognized end products in the body and the elimination of certain pigment complexes.

Any junior medical student can relate the true story of pigment metabolism in the human body. The medical textbooks have long retold the story as illustrated in Figure 1; and it seemed to be one of the facts in physiology which stood firm in the midst of progress and newer investigations. Its very age gave it respectability and true academic security of tenure. The time honored story is as follows: Certain food elements and iron are constructed in the bone marrow into a complex substance, hemoglobin. When the red cells are worn out or destroyed, the hemoglobin appears as bile pigment in a quantitative ratio. This bile pigment secreted into the intestine is changed to stercobilin and in large part excreted in the feces. Some of it may be absorbed and re-excreted by the liver; but, given a liver abnormality, it mav escape the portal blood stream and be excreted by the kidneys as urobilin.

A number of recent investigations have modified this picture somewhat, as illustrated by Figure 2. Wilbur and Addis [1] suggest that with the absorption of stercobilin the "pyrrol complex" may be split off and reconstructed into hemoglobin. This is a very interesting hypothesis and would be an example of a very pretty conservation on the part of the body, but we shall review experimental observations which we believe rule out this suggestion.

Our conception of body pigment metabolism may, perhaps, be expressed diagrammatically as shown in Figure 3. One point in particular deserves notice in that we no not accept as proved that there is any absorption of stercobilin from the intestine. Granting

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* From the George Williams Hooper Foundation for Medical Research, University of California, San Francisco; Harvey Society Lecture, Jan. 7, 1922.

[1] Wilbur and Addis: Arch. Int. Med. **13**:235 (Feb.) 1914.

that urobilin may be formed in the liver, there is not a shred of evidence, clinical or experimental, that stercobilin is ever absorbed from the intestine. Most observers admit that a times urobilin may be formed in the liver (Wilbur, Addis and many others), but forget this fact when absorbed in a discussion of the formation of stercobilin in the lumen of the intestine. We believe that stercobilin in the intestine is as little concerned with this question of pigment metabolism as is the stercobilin in the feces or the urobilin in the bladder urine. Therefore in Figure 3 we know of no evidence for a line between the circles indicating urobilin and stercobilin.

We have published (Whipple & Hooper[2]) evidence that bile pigment is not necessarily related directly to destruction of red cells and hemoglobin. This is indicated in Figure 3 by a direct line from "pigment complex" to "bile pigment" and illustrates the relation of bile pigment production to food and other factors which can modify pigment production quite apart from hemoglobin destruction. Granting that bile pigment production may be influenced by other factors than hemoglobin destruction, we see how absurd it is to draw conclusions unreservedly as to blood destruction from the analysis of stercobilin—for example, in pernicious anemia. The life cycle of the red cell has been established (Eppinger and Charnas[3]) on such flimsy evidence as the analysis of stercobilin.

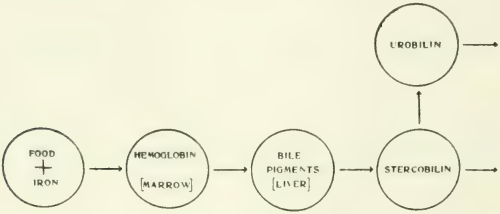

Fig. 1.—Pigment metabolism in the human body as formerly believed to occur.

That body protein as well as food factors are concerned in the production of bile pigment and hemoglobin is easily established by fasting experiments. Bile pigment excretion will continue during fasting periods, and, more than this, we have shown that hemoglobin will be formed in fasting periods not only sufficient for red cell maintenance but for actual increase above a moderate anemia level.[4] It is obvious that disintegration products of body cells are used in the upbuilding of hemoglobin and are related to the output of bile pigment, urobilin, stercobilin, and urochrome.

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[2] Whipple and Hooper: Am. J. Physiol. **40**:349, 1916.

[3] Eppinger and Charnas: Int. of klin. Med., 1913, p. 78.

[4] Whipple and Hooper: Am. J. Physiol. **45**:576, 1918.

BILE PIGMENT

Much of the older work on the biliary pigments has been reviewed recently by Hooper and Whipple[5] and need not be discussed at this time. It has been claimed by some that hemoglobin introduced into the blood stream will be quantitatively excreted as bilirubin in the bile. Whipple and Hooper[6] have been able to show that no such quantitative relationship holds for hemoglobin and bile pigment. It seems very probable that much of the hemoglobin set free in the blood stream may be used in the body economy for a variety of purposes—among others the construction of hemoglobin for new red cells. For example, it is sufficiently well established that intravenous injections of hemoglobin or the destruction of red cells in the body will aid in the recovery from simple anemia with consequent upbuilding of new hemoglobin.[7] It is very probable, however, that the hemoglobin in the blood stream is not used direct but only after being broken down to the unit structural factors—whatever these may be. This point is graphically illustrated by the double arrows between the "pigment complex" and "hemoglobin" in Figure 3.

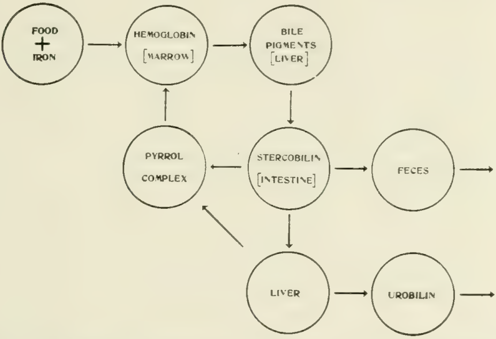

Fig. 2.—Pigment metabolism as modified by recent investigation.

It is now generally accepted that true bile pigment can be formed from hemoglobin within the body or by other than liver cells. Whipple and Hooper[8] showed that this transformation could be effected within two hours in the blood stream of the head and thorax with complete liver exclusion. The same workers[9] showed that hemoglobin can be transformed into bilirubin in the serous cavities within a period of twelve hours. McNee[10] has confirmed a part of this work. It is probable that the vessel endothelium and Kupffer cells are concerned in the vascular reaction. We believe that this reaction is not a physiologic curiosity but one of considerable importance in all conditions associated with escape of hemoglobin into the blood stream—for example, paroxysmal hemoglobinuria, malaria, toxic anemias, etc. Under such conditions we believe there is good evidence that much of the hemoglobin is changed to bile pigment and other substances quite apart from essential liver cell activity.

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[5] Hooper and Whipple: Am. J. Physiol. **40**:332, 1916.

[6] Whipple and Hooper: Am. J. Physiol. **43**:258, 1917.

[7] Hooper, Robscheit and Whipple: Am. J. Physiol. **53**:263, 1920. Itami: Arch. f. exper. Path. u. Pharmakol. **62**:104, 1910. Itami and Pratt: Biochem. Ztg. **18**:302, 1909.

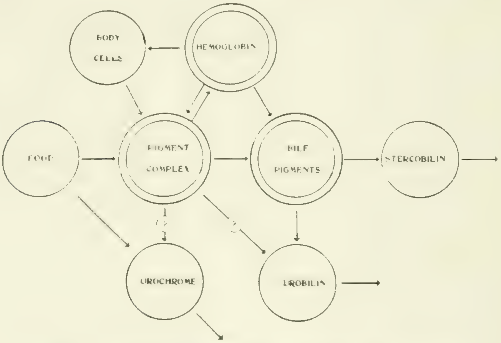

Fig. 3.—Conception of body pigment metabolism.

It is well established[2] that the bile pigment elimination in dogs can be increased by a change in diet—for example, a sudden change from a meat to a carbohydrate diet may increase the bile pigment elimination more than 50 per cent. This can be repeated time after time and it seems at least improbable that this reaction is dependent on blood destruction. We may explain this reaction, in part, as follows. The meat diet is normal for the dog. The increase in bile pigment excretion due to carbohydrate excess may represent an abnormal or alternative reaction—a deviation of pigment elements and construction into bile pigment for elimination. It is possible that some of these pigment elements concerned in this reaction might be

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8. Whipple and Hooper: J. Exper. M. **17**:612, 1913.
9. Hooper and Whipple: J. Exper. M. **23**:137, 1916.
10. McNee: J. Path. & Bacteriol. **18**:325, 1913.

available under favorable conditions (anemia) for hemoglobin construction or under usual conditions (meat diet) for elimination elsewhere than in the bile. Urochrome is a possible end product of pigment elements and deserves much more study in normal and abnormal conditions.

The term "pigment complex" is used in this paper to indicate a group of substances which are essential parts of the mature body pigments. It is obvious that certain food factors contribute to this "pigment complex" as foods are directly concerned in the production of new hemoglobin and the formation of bile pigments and urochrome. It is equally clear that the body protein and cells contribute to this "pigment complex," as all body pigments are produced in measurable amounts in fasting periods. We believe that the evidence is sufficient to show that as hemoglobin disintegrates in the body it also contributes to the "pigment complex" and so influences, in a measure, the new formation of hemoglobin. It is probable that only a small amount of the destroyed hemoglobin is conserved in this fashion. The pyrrol nucleus seems to be one of the factors which must be concerned in this "pigment complex," and it is probable that all facts related to pyrrol metabolism will have a direct relation to the complicated body pigment metabolism. These points are illustrated graphically in Figure 3.

Bile pigments in the bile fistula animal are not increased by the feeding of fresh bile pigments or of fresh or cooked blood or of digestion products obtained from blood.[11] This might be assumed to be from lack of absorption. There is no evidence that bile pigment or stercobilin are absorbed from the intestine. However, it has been shown that the feeding of hemoglobin will influence the curve of new hemoglobin construction after anemias.[7] This indicates an absorption of substances which are concerned with the "pigment complex" but it is clear that these same factors do not influence the output of bile pigments in bile fistula dogs—at least under the conditions of our experiments. These experiments are much against the suggestion of Addis (Fig. 2) that there may be a conservation of bile pigment factors which are absorbed from the intestine and reconstructed into hemoglobin. Perhaps the strongest argument against the absorption of stercobilin and its utilization in body pigment construction (Fig. 2) is the fact that bile fistula dogs under observation continuously for two years or longer show no evidence of pigment lack, no anemia, no fall in pigment production and no reaction whatever to the feeding of bile pigments.

A study of the bile pigment output of the Eck fistula liver furnishes some interesting facts to consider at this time. Dogs with combined Eck and bile fistulas eliminate less bile pigment than controls—sometimes only from 30 to 50 per cent. of normal.[12] The Eck fistula liver is functionally inefficient and there is no direct contact with the portal blood. Both these facts may well contribute to this low pigment output, but the main point to emphasize is that the pigment output is influenced by liver function rather than by the amount of hemoglobin waste products formed in the body. We have ample evidence that various liver injuries will likewise depress bile pigment excretion—again, clear evidence that the liver has a constructive function in producing bile pigments rather than a simple passive eliminative function.

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[11] Whipple and Hooper: Am. J. Physiol. **42**:256, 1917.

Bile fistula dogs with anemia give very interesting and complex reactions to hemoglobin injections.[6] In general, we may say that the elimination of bile pigments is not in any way parallel to the amount of hemoglobin injected. The same is true for control dogs with bile fistulas but no anemia. There is some evidence in these anemia experiments for conservation of certain of these pigment factors within the body. We may suspect a reconstruction of some of these factors into hemoglobin because of the anemia needs. There are some experimental data in favor of this explanation.

Certain of our bile fistula dogs have developed peculiar diseased conditions which may or may not be concerned directly with pigment metabolism. For example, certain dogs lose great amounts of inorganic salts from the bones, so much so that the ribs show very many "green stick fractures." These bones are reduced to mere elastic shells and the heavy long bones are likewise depleted of lime salts. There are many suggestive points in these experiments which are of peculiar interest at this time (diet and bony changes) but a discussion of this complex question must be postponed for the present.

Splenectomy in bile fistula dogs [13] gives reactions at times which are of the greatest interest to the hematologist. These dogs may show blood crises much like those observed in pernicious anemia. The color index may be very high for considerable periods—a most unusual condition in dogs. At times such dogs show maximum pigment production, which cannot be explained by destruction of red cells and hemoglobin. A specific experiment [Dogs 16-41, Table 69 [13]] shows periods of great bile pigment increase—even six times normal per kilo body weight. To account for all this bile pigment as derived from hemoglobin we must postulate a complete destruction of all circulating hemoglobin every four or five days or less, or a daily red cell replacement of from 20 to 25 per cent. This is unthinkable in the light of our knowledge of red blood cell regeneration in the dog, and we cannot imagine that all this pigment had been built up to hemoglobin before being broken down into bilirubin. Rather we wish to assume that the body is stimulated to a maximum production of pigment substances—in part, toward hemoglobin, in part, to bilirubin and, perhaps, to other pigment substances. The high color index indicates a maximum saturation of corpuscles with the pigment hemoglobin (refer to pernicious anemia below).

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[12] Whipple and Hooper: Am. J. Physiol. **42**:544, 1917.

[13] Hooper and Whipple: Am. J. Physiol. **43**:275, 1917.

Study of the bile pigments present in human serum in health and disease has been reported by a number of investigators (Van den Bergh,[14] Brulé,[15] Blankenhorn[16] and many others). It is evident that there are various free and partially bound pigments in the blood plasma or serum. Some of these pigments may dialyze through a parchment membrane and others will not do so. The significance of these various types of pigments has been discussed at considerable length by the different workers, but as yet there does not seem to be complete accord as to interpretations. Much important information will undoubtedly come from this work but wherever possible a more extended study of the body, feces and urine pigments should be made simultaneously.

UROBILIN

In discussing the pigment substances in the urine we wish to use the term urobilin to include the closely related substance urobilinogen. We use the term stercobilin to indicate the same substances in the feces. It seems to be accepted generally that stercobilin is formed in the intestine due to bacterial action on bile pigments. It is assumed solely on indirect and incomplete evidence that stercobilin is, in part, absorbed from the intestine. We believe there is no evidence for this assumption and much against it. This absorption of stercobilin should be discredited until such time as some positive evidence of intestinal absorption is brought forward. It is admitted by most clinical observers that urobilin is present at times in human bile in the liver[1] or gallbladder.[17] We have observed the presence of urobilin frequently in bile fistula dogs during fasting periods—in fact, this may seriously interfere with experimental work on bilirubin.[18] We believe that all evidence favors the production of urobilin in the liver and bile passages (cholangitis), its absorption at times into the blood stream from the liver and subsequent appearance in the urine. It is probable that

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14. Van den Bergh: Der Gallenfarbstoff im Blute, 1918.
15. Brulé: Recherchés récentes sur les icterus, Paris, Masson et Cie, 1919.
16. Blankenhorn: Arch. Int. Med. **27**:131 (Jan.) 1921.
17. Straus and Hahn: München. med. Wchnschr. **67**:1286, 1920.
18. Whipple, Hooper and Robscheit: Am. J. Physiol. **53**:151, 167, 1920.

urobilin, like bilirubin, at times is produced in tissues other than the liver (blood extravasations, pneumonia, etc.).

We do not wish to deny the value of urobilin determination on the urine, feces and bile, but the deductions drawn from such observations should be made with a proper conception of the various reactions concerned; and absorption of stercobilin is not one of the factors to be accepted at present. Studies of urobilin in the urine may indicate certain liver abnormalities or pigment disturbances in other parts of the body. Analyses of stercobilin give figures of great interest but high figures may indicate overactivity of the liver in pigment production rather than excessive blood destruction with elimination through the liver of bile pigments or urobilin. The analysis of fresh human bile obtained by means of the duodenal tube will give facts of much value (Lyon and others [19]). It should be remembered that such samples are suitable only for qualitative analysis. The amount of dilution can never be determined but the ratio of bile pigments to bile acids may be of considerable significance, although difficulties in analysis are present. The presence of urobilin and abnormal elements in the duodenal bile may contribute facts of great value for a complete understanding of a complicated disease picture.

The various methods for determination of urobilin or stercobilin are admittedly unsatisfactory. Moreover, certain changes in the feces may make analysis inaccurate or quite impossible (constipation, diarrhea, etc.[1]). Attempts aimed toward accurate quantitative extraction are reported by Hausmann,[20] Goiffon,[21] Baumann [22] and others. When a simple and accurate quantitative method is at hand the work on these problems will be greatly facilitated. A method to give complete satisfaction in stercobilin estimations must give accurate figures not only for stercobilin but its various related substances which confuse the picture in simple complications like constipation and diarrhea.

UROCHROME

Urochrome is the stepchild of the pigment family. Numerous theories as to its origin have been advanced but it can safely be asserted that its parentage is doubtful. It has been suspected that urochrome was related to urobilin, to blood pigments, to urea and to food pigments. These views have recently been reviewed by Pelkan,[23] whose work indicates an important relationship of urochrome to food protein. He brings evidence that carotin is not concerned in the urochrome excretion.

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[19] Lyon: J. A. M. A. **73**:980 (Sept. 27) 1919.

[20] Hausmann: Ztschr. f. exper. Path. u. Therap. **13**:373, 1913.

[21] Goiffon: J. Pharmacol. Chem. **21**:236, 1920.

[22] Baumann: Arch. Int. Med. **28**:475 (Oct.) 1921.

[23] Pelkan: J. Biol. Chem. **43**:237, 1920.

Urochrome contains the pyrrol group,[24] which is always of interest in any pigment work. There has been no extensive work done to study the appearance and relationship of this pigment with other important body pigments in health and disease. It is at least possible that urochrome forms one of the avenues of disposal of pigment elements ("pigment complex") even before such substances are built into hemoglobin or bile pigments. These possibilities do not preclude fluctuations of urochrome due to large intake of foods rich in pigment forming materials. This substance should be studied simultaneously with other pigments (blood and bile) in health and disease, in clinical and experimental conditions.

LIPOCHROME

Lipochrome is a peculiar pigment which is thought to have merely a passive function in the body with no relationship to the urobilin, urochrome or other pigments containing the pyrrol nucleus. Schulze[25] states that lipochromes are very closely related to the yellow radicle of chlorophyll and members of the group of carotinoid pigments including carotin and xanthophyll. The lipochromes are soluble in fat and fat solvents. Dolley and Guthrie[26] state that these lipochromes can be removed from the body fat almost completely by diet periods free from carotin intake. Van den Bergh[14] states that the lipochrome content of the blood varies with the diet intake of these food pigments. It is well known that the serum of diabetics is rich in lipochrome pigment, and he attributes this to diet factors. At present we have no reason to suppose that the lipochromes have any direct relationship to the other body pigments, but we should not close our minds to this possibility, especially in disease conditions, for example, hemochromatosis and pernicious anemia.

HEMOGLOBIN

Hemoglobin is without question the most important and interesting of all the body pigments. Much of the interest in other body pigments comes from a relationship known or assumed to exist between these various pigments and hemoglobin. A very closely related or identical substance, myohematin (muscle hemoglobin) exists in the striated muscle tissue of the body and undoubtedly plays an important part in the rapid exchange of oxygen and carbon dioxid between the functioning muscle protoplasm and the circulating hemoglobin. Myohematin will not be considered in detail in this paper, but we should always keep this substance in mind when we study various gases (for example, carbon monoxid) which are absorbed by hemoglobin in the body. The genesis of myohematin is of fundamental importance, and we have no right to assume that the bone marrow cells are concerned in its production. Is this substance formed from its very elements by the activity of the muscle tissue alone? Or is some partially built up "pigment complex" utilized by the muscle tissue in building this most complex substance? How rapidly is the myohematin used up in the body as compared with the daily wastage and repair of circulating hemoglobin? It will be of great importance to study various conditions in which myohematin may be present in abnormal amounts. It has been pointed out recently [27] that this substance usually amounts to about 10 per cent. of the total body hemoglobin, but much more work is needed to ascertain the conditions under which the myohematin content may be found to depart from normal. Such studies will give information of much value for the complete understanding of the body pigment metabolism. Hoagland [28] has recently completed experiments to show that aseptic anaerobic autolysis of beef muscle will produce measurable amounts of hematoporphyrin. He suggests that this is a normal reduction product of hemoglobin in the body and a substance intermediary to the final end product, bilirubin.

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[24] Weiss: Med. Klin. **13**:659, 1917.

[25] Schulze: Sitz. Ges. Nat. Freunde. Berlin. 1914, p. 398.

[26] Dolley and Guthrie: Science **50**:191, 1919.

In the adult human it is generally assumed that hemoglobin is fabricated in the protoplasm of red cell groups within the bone marrow. We have suggested that other tissues (for example, the liver) may be concerned in building up "parent pigment substances" which are essential to the proper construction of mature red cells containing hemoglobin. It is worth while reviewing a few facts concerning the development of red cells in the embryo. Sabin [29] has pointed out that blood cells in the second day chick embryo develop from the endothelial cells and angioblasts. Hemoglobin is present at this time but the liver is not functionally active. This seems at first sight to indicate that endothelial cells can produce red cells and the pigment hemoglobin. This may in fact be true but we must not forget the yolk sac with its various storage factors developed by the mature hen and drawn on continuously by the developing embryo. The endothelial cell, however, may be active in a variety of the body pigment reactions and can almost certainly produce bilirubin from hemoglobin.[8]

Therefore, it is possible that these cells have a capacity to build up pigment substances and so take part in hemoglobin production. We recall, too, the interesting relationship in early fetal life (human)

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27. Smith, Arnold and Whipple: Am. J. Physiol. **56**:336, 1921.
28. Hoagland: J. Agricul. Res. **7**:41, 1916.
29. Sabin: Anat. Rec. **13**:199, 1917.

between the liver cells and the islands of blood forming cells. This would indicate a possible relationship between the liver cell, the Kupffer cell and the developing red blood cell. It is, of course, possible that this relationship continues through the agency of the circulation during adult life.

DIET FACTORS AND HEMOGLOBIN

We have long been interested in the influence of various diet factors on the regeneration of red cells and hemoglobin during periods of simple anemia in dogs experimentally produced by hemorrhage. This work was begun by C. W. Hooper and me as an outgrowth of our bile pigment investigations and later continued with the cooperation of F. S. Robbins. The investigation is now being continued by Robbins and Whipple. We may refer to certain publications [18] for much of the experimental detail, methods, protocols, etc. At the outset it seemed obvious from simple experiments that hemoglobin regeneration could be influenced easily by a variety of diet factors. We thought it highly desirable to work with animals of sufficient size and suitable type so that blood could be obtained readily for various analyses by venous puncture. Dogs were obviously best suited for these experiments. The removal of small blood samples is very easy and does not complicate the regeneration curve as the amounts removed are so small as compared with the blood volume (from 800 to 1500 c.c.). These dogs are omnivorous and will eat readily all types of food mixtures. Our routine experiments included careful determinations of blood volume, hemoglobin and red cell hematocrit, red cell counts from blood drawn by venous puncture and body weight.

Our experimental data give numerous examples to show the necessity of such complete determinations and the mistaken deductions which may be derived from incomplete experiments. This applies particularly to experiments of long duration where the differences between any given group and the controls are but slight. The general procedure is as follows: A group (usually four) of normal dogs is standardized (blood volume, red cell hematocrit, red cell count, etc.). These dogs are then bled one-fourth of their total blood volume on two successive days, at times a third bleeding is used. After a rest of one day they are again standardized and placed on the experimental food mixture. Complete determinations are done thereafter each week until the end of the experiment.

Two characteristic tables (Tables 1 and 2) may be cited as examples of the wide differences in hemoglobin regeneration which may be associated with sugar feeding (Table 1) and with meat feeding (Table 2). We see that the blood regeneration is but slight—a slight increase over and above the maintenance factor in red cells and an

TABLE 1.—BLOOD REGENERATION AFTER SUGAR FEEDING

Dog 17-28. White bull, female, adult.

Date, 1917	Pigment Volume = Hb. per Cent. Times Blood Volume	Blood Volume	Plasma Volume	R. B. C. Volume	R. B. C. Hematocrit	Hb.	Color Index	Hb. Index	R. B. C., Million	W. B. C.	Weight	Blood per Kilogram	Remarks
		C.c.	C.c.	C.c.	%	%					Kg.	C.c.	
1/19	1.620	1,500	600	900	60.0	108	0.76	1.80	7,1	7,4	11.60	129	Fasting
1/20	Bled 375 c.c.												
1/22	Bled 270 c.c.												
1/23	Bled 105 c.c.												
1/24	588	900	603	306	34.0	61	0.80	1.79	3,8	7,3	10.40	87	
1/24	Diet: 50 gm. cane sugar, 25 gm. glucose, 400 c.c. water												
2/ 2	717	1,121	684	437	39.0	64	0.62	1.64	5,2	7,2	9.40	120	*Anis.
2/ 9	636	1,027	637	390	38.0	62	0.65	1.63	4,8	6,2	8.90	115	
2/16	634	961	586	375	39.0	66	0.59	1.69	5,6	10,0	8.50	113	Diarrh.+
2/23	541	933	562	373	40.0	58	0.56	1.45	5,2	9,0	8.00	117	

* Anisocytosis of red cells.
Blood volume with dry oxalate. Hemoglobin with Sahli tubes.
Hemoglobin index equals hemoglobin per cent. divided by red cell hematocrit per cent.

TABLE 2.—BLOOD REGENERATION AFTER BEEF HEART AND LIVER

Dog 18-116. Bull mongrel, female, young adult.

Date, 1918-1919	Pigment Volume = Hb. per Cent. Times Blood Volume	Blood Volume	Plasma Volume	R. B. C. Volume	R. B. C. Hematocrit	Hb.	Color Index	Hb. Index	R. B. C., Million	W. B. C.	Weight	Blood per Kilogram	Remarks
		C.c.	C.c.	C.c.	%	%					Kg.	C.c.	
12/ 2	2,120	1,720	805	907	52.7	123	0.56	2.32	11,4	1,14	14.90	115	
12/ 2	Diet: Crackermeal and milk												
12/ 2	Bled 430 c.c.												
12/ 4	Bled 430 c.c.												
12/ 6	939	1,182	803	369	31.2	79	0.57	2.54	6.9	21,2	14 55	81	
12/ 7	Bled 300 c.c.												
12/ 9	776	1,260	930	323	25.6	62	0.84	2.38	3,7	17,5	13.95	90	
12/ 9	Diet: 256 gm. cooked beef heart;* 610 gm. cooked beef liver*—100 calories per kilo												
12/16	1,082	1,330	844	476	35.8	81	0.88	2.25	4,6	18,2	14.55	91	
12/23	1.890	1,685	860	818	48.5	112	0.57	2.30	9,9	11,5	15.25	110	
12/30	2,270	1,648	747	902	54.7	138	....	2.50	....	....	15.50	106	

* Meat cooked, fat and connective tissue removed and ground.
Hemoglobin index equals hemoglobin per cent. divided by red cell hematocrit per cent.

even smaller increase in hemoglobin within a period of four weeks. The contrast with meat and liver feeding is striking (Table 2) which shows complete return to normal in a period of three weeks. Between these extremes are all types of reaction, and it is easy to understand that a diet containing several food factors may give a complicated reaction. It is easy to understand that one food factor may be inert in this reaction and a second factor may likewise be inert but the two together may have a distinct influence on the curve of red cell and hemoglobin regeneration. For this reason it is necessary to test a given factor under a variety of conditions (supplementary feeding, etc.) before we can feel sure that we understand its reaction under anemia conditions. This is one of several reasons why the accumulation of convincing experimental data is so time consuming. We cannot accept the experiments of Downs and Eddy,[30] who report a positive influence of secretin on the production of red cells in anemia. They record only the red cell count with no figures for red cell hematocrit, hemoglobin or blood volume observations. That secretin may influence the red cell and hemoglobin production may be true but this is neither proved nor disproved by their experiments.

MEAT

Our experiments show that diets of cooked beef muscle or cooked beef heart are very favorable for a rapid regeneration of red cells and hemoglobin (Table 2 and others[31]). Cooked liver ranks with cooked muscle, and these food factors will effect a prompt reconstruction of the anemia picture to normal. These favorable diet factors are also potent when given after long periods of anemia and unfavorable diet intake. This is the severest test for any diet factor as to its influence on red cell and hemoglobin reconstruction. Certain diet factors may give a favorable reaction if given at once after the anemia is produced but may give an unfavorable reaction if given at the end of a long period of anemia and unfavorable diet intake.[31] Meat extracts (commercial) are inert and possess none of the factors which influence red cell and hemoglobin regeneration.

FOOD GRAINS

The common food grains (wheat, barley, rice) in the form of cooked bread or crackers do not furnish many factors which promote red cell regeneration. Full diets of these materials with skim milk may effect a slow rise in the level of hemoglobin and red cells which finally may reach normal in from six to eight weeks. More com-

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30. Downs and Eddy: Am. J. Physiol. **58**:298, 1921.
31. Whipple, Robscheit and Hooper: Am. J. Physiol. **53**:236, 1920.

monly the return toward normal will not exceed 90 per cent. of the initial level before the anemia is produced by bleeding. Casein and skim milk may be ranked with these food grains as regards their influence on hemoglobin regeneration and we may say that, as a rule, these foods do not return anemia animals to a high red cell and hemoglobin level.

FASTING

A comparison of anemia fasting experiments with sugar feeding experiments shows that anemic dogs actually produce more red cells and hemoglobin during fasting periods than during periods of sugar feeding. The question of the "sparing action" of carbohydrates comes into this reaction, but we must refer the reader to reviews [18] of this subject in recent papers.[32] There is evidence from these experiments that the body conserves with much care the various pigment construction units, which are then recast into red cells and hemoglobin. It is well to keep in mind the fact that normal dogs during periods of zero nitrogen intake (fasting or sugar feeding) are able to form hemoglobin and red cells over and above the considerable amount needed for daily wastage and repair—also are excreting considerable amounts of bilirubin, stercobilin and urochrome. These must all come directly or indirectly from the host's protein. This careful conservation of red cell and hemoglobin construction factors must be of considerable importance in the body economy.

IRON

Iron furnishes a never failing topic for discussion by internist and physiologist alike. Clinical opinion favors the use of iron in simple anemias, but we question whether this treatment is based on sound evidence. At least, the treatment does the patient no harm and may soothe the doctor's conscience, but we can find no convincing evidence that it gives patients with secondary anemia any real benefit. This discussion does not concern chlorosis, which appears to be a distinct disease entity. All the experimental evidence indicates that iron is inert in secondary anemias and has no influence on blood regeneration. Our experiments [33] indicate that iron in the form of Blaud's pills is inert.[7] Ferric citrate and ovoferrin have little or no influence on blood regeneration—nothing to compare with favorable food factors (meat). Hemoglobin, given by mouth, intraperitoneally or intravenously, does influence the curve of red cell and hemoglobin regeneration in a positive fashion but not to the extent noted with potent diet factors (meat). The iron may be concerned in this reaction but there is even more evidence in favor of the pyrrol complex. Likewise, arsenic in sodium cacodylate or Fowler's solution is inert in these anemia periods. We believe it will repay the clinical workers to record careful observations on anemic patients, paying particular attention to various diet factors proven to be potent in controlled experiments.

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[32] Davis and Whipple: Arch. Int. Med. **23**:689 (May) 1919.

[33] Whipple and Robscheit: Arch. Int. Med. **27**:591 (May) 1921.

The relation of hemoglobin and chlorophyll has given rise to much speculation in the past and there are many interesting possibilities which call for more work. It has been pointed out that under usual conditions iron is necessary in plant metabolism for chlorophyll production but does not form a part of the chlorophyll nucleus.[34] In the absence of iron plants can form chlorophyll if there is a supply of pyrrol material.[35] These observations are of particular interest when we consider the influence of certain plant leaves rich in chlorophyll on the blood regeneration of anemia dogs (see below). But the evidence that iron is directly concerned in this reaction is not convincing.

It has been reported by Cloetta [36] that the iron present in hemoglobin is not absorbed from the dog's intestinal tract, and he is able to recover the iron quantitatively after blood feeding. Weber [37] has reported experiments to indicate that iron lactate is inert in anemia periods in experimental animals. However, he did not control his diet and reports on hemoglobin values only. The anemia was produced by pyrodin. His experiments are not convincing but give no evidence that this iron salt is potent. Musser [38] has furnished more evidence that iron is inert under experimental conditions. Iron was given as ferrous sulphate in capsules and the dogs rendered anemic by repeated bleedings. He gives complete data on hemoglobin, red cell count, resistance of red cells to hypotonic solutions and blood volume. It is, perhaps, unfortunate that a mixed diet of hospital food scraps was used in all these experiments. As diet factors are important it is very desirable that the investigator knows the amount and type of food intake. A mixed diet gives an opportunity for much variety and also choice by the animal. As a rule, the blood regeneration is very rapid on a mixed diet and gives less opportunity to demonstrate the influence of any given factor on blood regeneration. Some experiments should be included to show the influence of the given drug when administered during a long period of feeding on a diet unfavorable to rapid blood regeneration—for example, a 75 calory per kilo diet of bread and skim milk.

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34. Moore: Proc. Roy. Soc. Lond. **87**:556, 1914.
35. Oddo and Pollacci: Gazz. Chim. Ital. **50**:54, 1920.
36. Cloetta: Arch. f. exper. Path. **37**:69, 1895.
37. Weber: Ztschr. f. Biol. **70**:168, 1920.
38. Musser: Arch. Int. Med. **28**:638, 1921.

FATS

Unpublished experiments of Robbins and Whipple indicate that lard is inert in various diets, and influences in no degree the regeneration of red cells and hemoglobin in anemic dogs. Numerous experiments with cod liver oil give no evidence that this oil influences blood regeneration. In striking contrast stand the experiments with butter fat, which indicate that under certain conditions some substance in butter fat is able to influence in a striking way the curve of hemoglobin regeneration and hasten the production of hemoglobin and red cells. When we admit that butter feeding is potent in anemia regeneration of hemoglobin, we can scarcely admit that it actually is built into the hemoglobin molecule. But it may act in some way to facilitate the linkage of the various complexes which go into the large hemoglobin molecule. We have not as yet sufficient data on the influence of butter feeding upon the output of other body pigments during anemia periods.

FISH

Through the friendly cooperation of the California Packing Corporation and the Alaska Packing Corporation we were able to test a variety of food fish. These experiments will be published in the near future. The highly pigmented cooked salmon muscle was used and in a variety of diet tests was shown to be inert in anemia experiments. It is of particular importance to note that this muscle pigment of the salmon is not concerned in the upbuilding of hemoglobin in dogs. We have some evidence that the myohematin of beef muscle is actually concerned in hemoglobin regeneration, which is so rapid after beef feeding. Whale meat was tested and found to act exactly like beef muscle or other striated mammalian muscle. Clams, like fish, are inert in anemia experiments.

VEGETABLES

The data concerning vegetables will be published shortly by Robbins and Whipple. A number of vegetables have no effect on the curve of red cell and hemoglobin regeneration after anemia. Carrots are inert and deserve particular attention because of their high content of carotin, a food pigment which does enter into the body fluids and tissues. Dehydrated celery, parsley and Brussels sprouts are likewise inert, and it is improbable that the dehydration was responsible for the negative reaction as dehydrated spinach is positive and exerts its usual influence on the hemoglobin reaction. Fresh beet tops are negative, as compared with fresh spinach, which is positive and even more potent than the dried spinach meal. Canned spinach is somewhat less potent than is the freshly cooked material. These observations show that by this physiologic test there is a distinct difference between the chlorophyll of sprouts, celery, parsley, and beet leaves as contrasted with the chlorophyll of spinach.

PERNICIOUS ANEMIA

This is a diseased condition, little enough understood, which is of the greatest interest to any person investigating general body pigment metabolism. Let us examine the facts as known, paying particular attention to this question of pigment metabolism. We find an excess of pigment substances everywhere in the body—increase of pigments in the liver cells, blood stream, bone marrow, feces and, at times, in the urine. There is a high hemoglobin index or we may say the red cells are saturated with hemoglobin as contrasted with the 50 per cent. hemoglobin content of a simple anemia. This suggests that the body has an excess of pigment material available and is producing various pigment substances at an abnormal rate of speed—compare the experiment (the splenectomy bile fistula dog, No. 16-41 [13]) which showed a high color index and enormous overproduction of bile pigment.

We are told that the stercobilin analysis in a case of pernicious anemia is an index of blood destruction. Let us examine some of these figures and, further, let us assume the normal red cell count as 5,000,000 and the pernicious anemia count as 1,000,000 for the sake of simplicity of comparison. If the anemia red cells disintegrate at the same rate of speed as the normal control, and if these products result in bilirubin and then stercobilin we must say the stercobilin figures should be one-fifth of normal. But the stercobilin figures during periods of remission in pernicious anemia often exceed twice or three times normal stercobilin excretion, making no allowance for similar pigments in the urine. This can only mean that the pernicious anemia patient with one-fifth the number of red cells and two or three times the amount of stercobilin output must regenerate its total red cell mass every three days instead of the assumed normal of every thirty days. The normal replacement factor for red cells and hemoglobin is believed to be 3 per cent. per day.[39] We must postulate from 30 to 40 per cent. replacement of red cells per day in a pernicious anemia case if we persist in explaining the stercobilin content as being due to blood destruction. Those who wish to accept this explanation are welcome to do so, but it would be a fleeting and troublous life period endured by the red cell in pernicious anemia!

Ashby [40] has published recent observations to show that the life cycle of the human red cell is variable and may be thirty days or at times 100 days. Further than this she submits observations which indicate that in pernicious anemia the red cells exist in the circulation at least as long as in the normal human case and perhaps for a longer time. She questions the importance of an increased blood destruction in producing the anemia of this disease.

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[39] Ashby: Jour Exper. M. **29**:267, 1919.

[40] Ashby: J. Exper. M. **34**:127, 1921.

We prefer to explain the observed facts as done in Figure 3, assuming that there is a great stimulus within the body for pigment production. It may well be that there is an overproduction of pigments, including hemoglobin and bile pigments and other abnormal pigments. For example, in certain cases of pernicious anemia Hooper and Whipple have observed an unusual pigment in gallbladder bile obtained at necropsy—a pigment which must be treated with active acetaldehyd before it will give the usual bile pigment tests. It is highly probable that this pigment, after a stay in the intestine, would be reduced to stercobilin and take part in the familiar high reading of stercobilin in such cases.

Our conception of pernicious anemia is that there is a scarcity of stroma building material or a disease of the stroma forming cells of the marrow which limits the output of red cell framework. There is plenty of pigment material (an excess in fact) as evidenced by the high color index or the saturation of the red cell with hemoglobin. Wherever we meet with a high color index we should suspect some deficiency in stroma construction or some overproduction of body pigments including hemoglobin. Conditions of malignancy, for example, with the hematology of pernicious anemia should yield information of value when examined with these points in mind. Nothing in this paper should be construed as minimizing the importance of stercobilin analyses for we are confident that such information is of great value. High stercobilin figures may be a very valuable diagnostic aid in obscure cases of pernicious anemia, as claimed by Hausmann and Howard.[41] That these figures indicate a corresponding destruction of red cells may be doubted and an overproduction of pigment may be a safer assumption.

Under usual secondary anemia conditions we know that this physiologic anemia stimulus causes a rapid production of both stroma and hemoglobin, the production of the stroma even outstripping that of the hemoglobin, as indicated by the low color index. It is possible that stroma production in the body may be fatigued more easily, as suggested by certain anemia conditions of long standing, which may show periods of high color index and a hematology suggesting pernicious anemia. Such cases are deserving of particular attention and a most careful study of the complete pigment metabolism.

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[41] Hausmann and Howard: J. A. M. A. **73**:1262 (Oct. 25) 1919.

The experiments of McMaster and Haessler[42] may have an interesting bearing on some of these questions. They show that in rabbits the injection of hemoglobin intravenously is a stimulus to the extension of bone marrow tissue in the long bones of the experimental animals. Simple bleeding does not stimulate to the same degree this "spread" of the bone marrow. They believe that there is no evidence for direct utilization of the injected hemoglobin, which is probably broken down before use. So it is possible that the pigment construction factors or "pigment complex" may be radicles which determine the bone marrow spread.

HEMOCHROMATOSIS

This disease is characterized by abnormal deposits of various pigments in many body cells and tissues. By most writers it is thought that this increase in body pigments is to be explained by a great increase in blood destruction. The mere fact that there is no evidence to support his hypothetical increased blood destruction has not deterred writer after writer from this assumption. Sprunt[43] is probably the first writer to object to this interpretation, and he points out convincingly the entire lack of evidence for any increase in red cell destruction—further that the evidence favors some profound disturbance in body metabolism which permits the deposit or production of various pigments in the protoplasm of various body cells. MacCallum[44] has shown the presence of iron in the cystoplasm of ferment forming gland cells of all descriptions. It is interesting to recall the presence of abnormal iron containing pigments in these same cells in cases of hemochromatosis. The liver and pancreas are especially involved in this reaction, and the liver may contain 100 times the normal content of iron. Various observers record normal stercobilin elimination in cases of hemochromatosis. Wilbur and Addis[1] report a long series of observations on a case of hemochromatosis to show normal stercobilin figures and fluctuating amounts of urobilin in the urine. The necropsy disclosed cirrhosis of the liver and the familiar picture of this disease. It would be interesting to speculate about the pigment abnormalities in hemochromatosis, but we must recall that actual observed facts are but few in number. It can be said, however, that there is no evidence of an increase in red cell and hemoglobin destruction. Stercobilin elimination may be normal but we do not know anything as to iron intake and elimination. It will be of great interest to study the various pigment factors in the blood, urine and tissues. It is, at least, possible that the usual paths of pigment elimination and disposal are, in part, blocked at the source

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42. McMaster and Haessler: J. Exper. M. **34**:579, 1921.
43. Sprunt: Arch. Int. Med. **8**:75 (July) 1911.
44. MacCallum: Ergebn. d. Physiol. **7**:552, 1898.

so that these pigments which may be present in various cells in traces are unable to escape and heap up within the cell protoplasm. This explanation suggests that various cell protoplasm may at times be concerned with the building up of certain pigment substances rather than with a simple passive storage. We may choose to look on this disease as resembling diabetes in certain respects—one disease with an inability to handle the carbohydrates, the other disease associated with abnormal metabolism of pigment factors. Certain end products are toxic in the first instance (diabetes) and again other products accumulate in the body cells (hemochromatosis) to their detriment and final destruction.

In this connection we wish to refer to experiments of Rous and Oliver,[45] who induced plethora in rabbits by intravenous injection of red cells. After months these animals developed siderosis of the liver, kidney and other tissues. Their experimental findings in animals have many points of similarity to those observed in human cases of hemochromatosis. Their conception that the essential disease factor in hemochromatosis lies in the liver does not wholly satisfy the writer, but their experiments bring some support to the explanation advanced above in favor of a disturbance of the whole intracellular pigment mechanism.

CONCLUSION

In conclusion, we may repeat that the various body pigments have interesting relationships which, perhaps, are not as simple as commonly believed. There may be at times a parallel increase or decrease of related pigments. We wish to make clear our conception of an underlying "pigment complex" which may be an intermediary stage in the development of mature pigments. Perhaps all pigment construction units pass through this stage and, depending upon supply and demand, are used to construct hemoglobin or bile pigment or are discharged as urobilin or urochrome or related pigments, as illustrated in Figure 3.

What may be the fundamental stimulus for pigment manufacture in the body is for future work to determine. The most important single point which we wish to emphasize is that pigment production depends upon constructive body cell activity—a dynamic function which concerns the formation of hemoglobin and bile pigments as well as urobilin and urochrome.

We recall that the output of bile pigment in the dog may be influenced by a variety of food factors but not by the feeding of bile pigment or hemoglobin. Bile pigment output depends on liver

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45. Rous and Oliver: J. Exper. M. **28**:629, 1918.

functional activity, and we believe it is a product of liver activity not solely a passive elimination product coming from defunct hemoglobin. Splenectomy and bile fistula combined may give a maximum pigment output and a clinical picture resembling in some respects pernicious anemia. There is convincing evidence that stercobilin is not absorbed from the intestine and further that urobilin as observed in the urine is formed in the liver or body tissues, not absorbed from the intestine. Urochrome is a pigment too little studied. It may represent a shunt for pigment building material not utilized in the body. Lipochrome seems to be an inert pigment taken in with the food and slowly eliminated.

Hemoglobin regeneration following anemia may be influenced by a great variety of diet factors. Among the potent factors exerting a positive influence on hemoglobin stand, first, red meat and cooked liver, hemoglobin and butter fat. Then come spinach and full diets of common food grains and milk. Practically inert are other chlorophyll containing vegetable leaves—celery, parsley, beet tops and sprouts. In the same negative group are fish and clams, onions and beets and animal fats, including lard and cod liver oil. Iron and arsenic in the common drug preparations are likewise inert under these conditions.

Pernicious anemia and hemochromatosis show abnormalities of pigment formation and disposal. We believe these two diseases have this in common—a definite overproduction of pigment in the body but not an increased destruction of hemoglobin and red cells. These are pigment abnormalities which deserve the most careful study from this point of view. Such studies should be sufficiently comprehensive to include simultaneous observations of these pigment elements in the blood, feces and urine, also in the bile and body tissues whenever possible.

It may be that this paper is too speculative, but hypotheses are of some value for further work even if there be little or no truth in them. I hope to furnish more data in the near future bearing on these questions.

Reprinted from the Archives of Internal Medicine
June, 1922, Vol. 29, pp. 711-731

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Five Hundred and Thirty-Five North Dearborn Street
CHICAGO

SECOND PROGRESS REPORT ON THE INVESTIGATION OF THE CHEMOTHERAPEUTICS OF CHAULMOOGRA OIL AND ITS DERIVATIVES IN LEPROSY AND EXPERIMENTAL TUBERCULOSIS*

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In a first progress report, communicated to the National Tuberculosis Association last year, three main lines of investigation on this problem were purposed, namely: (1) the preparation of derivatives of chaulmoogra oil for therapeutic experiments; (2) a biochemical study of the fate of chaulmoogra oil and its derivatives in the animal body; and (3) the therapeutic action of chaulmoogra derivatives on experimental tuberculosis. A summary of the progress of the investigation along these three lines is presented in this report.

I. THE PREPARATION OF DERIVATIVES OF CHAULMOOGRA OIL

Of the ordinary compounds of the chaulmoogric acids, the sodium and potassium salts are water soluble and the series of esters are light fluids; all other compounds have been found to be insoluble and of a fatty or waxy nature and are consequently unsuitable for therapeutic use. The sodium and potassium salts, being soaps, are very irritating to the tissues. Special attention has been devoted to the preparation of the esters, on account of their thin fluid consistency and neutral reaction and because of the superior therapeutic results claimed for the ethyl esters in the treatment of leprosy.

The essential steps in the preparation of these esters are the separation of the fatty acids from chaulmoogra oil by saponification with hot alcohol-potash solution, the esterification of the fatty acids by passing dry hydrochloric acid-gas to saturation through a heated solution of the chaulmoogric acids in the appropriate alcohol under a reflux condenser and the purification of the esters by distillation at high temperature under vacuum. The benzyl ester, being decomposed by the high temperature of distillation, was purified by filtering through bone charcoal.

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* From the George Williams Hooper Foundation for Medical Research University of California Medical School, San Francisco.

Work done under grant of the Committee on Medical Research of the National Tuberculosis Association.

The different esters have been found to vary greatly in the reactions which they produce when injected into animals. Some of them, such as the methyl and the amyl esters, cause pain, oedema, hemorrhage and necrosis at the site of injection and systemic disturbance, as indicated by progressive loss of weight and condition of the animals, when given in repeated injections. These esters are wholly unsuitable for therapeutic use. The ethyl ester, now used so extensively in the treatment of leprosy, is open to the same objections of local and constitutional reactions, although to a lesser degree than the methyl and amyl esters. Tuberculous rabbits, treated with the ethyl ester in doses larger than 0.0715 cc. per kilo of body weight, develop so severe and persistent local reactions that treatment has to be discontinued. Leper patients under treatment with ethyl ester complain of pain and persistent soreness at the site of injection and some of them suffer so severe constitutional disturbance that continuation of the treatment becomes impossible. In contrast to these esters, two new esters, that we have prepared, the butyl and the propyl esters, produce practically no local reactions or systemic disturbance in experimental animals, even in double the standard therapeutic dose administered repeatedly over long periods of time. Trial of these new esters on a limited number of lepers has confirmed the absence of unpleasant reactions and has given as good therapeutic results as the ethyl ester. We believe these new esters to be superior for therapeutic purpose to any derivative of chaulmoogra oil hitherto produced.

Further work on the preparation of new derivatives has been discouraged by the results of our biochemical study of the fate of chaulmoogra derivatives in the animal body. We had hoped that some derivative could be produced which would be capable of circulating and penetrating the tubercular lesions unchanged; but our bio-chemical studies indicate that in whatever form or route the chaulmoogric acids are introduced into the body, the compound is hydrolized and the chaulmoogric acids are metabolized like ordinary fats. Therefore, the value of any new compound is reduced to the questions of its absorbability and freedom from toxic reactions. There remains, of course, the possibility of some chemical modification of the chaulmoogric acid molecule more profound than the substitution of the hydrogen atom in the carboxyl radical of the side chain, such as an elimination of a part or a whole of the side chain, or substitution in the ring, which might alter the physiological behavior without destroying its bactericidal property.

II. THE FATE OF CHAULMOOGRA OIL AND ITS DERIVATIVES IN THE ANIMAL BODY

In undertaking our biochemical study of chaulmoogra oil, we were confronted at the outset by the problem of methods of isolating and identifying minute quantities of the chaulmoogric acids in the presence of relatively large amounts of other fats in the animal body. There exist no precise methods of separating the different fatty acids nor delicate chemical tests for the individual fatty acids. While the fatty acids of the chaulmoogric series differ fundamentally in chemical structure from other fatty acids, we have been unable so far to devise a satisfactory chemical method for their separation and estimation. Fortunately, however, the chaulmoogric acids possess a physical peculiarity, optical activity, associated with their ring structure, which is not a property of other fatty acids. Making use of this optical activity, we have devised a technic by which, after appropriate chemical treatment of the tissues or body fluids, we are able to read quantitatively the chaulmoogric acids with the polariscope, in the presence of other fatty acids in the solution, in amounts as small as one milligram. This test, while not so delicate as might be desired, has proved fairly satisfactory in this investigation.

The first step in the study of the fate of chaulmoogra oil and its derivatives in the animal body has been to determine their absorption by different routes. When ingested, the absorption of the chaulmoogric acids from the alimentary tract has been found to be as complete as is the absorption of other fats, namely, from 95 to 98 per cent. of the ingested fatty acids. From the completeness and rapidity of the absorption by this route, it would appear that the old method of treating leprosy by ingestion of the crude chaulmoogra oil should be as effective, except for the gastric disturbances, as the newer method of intramuscular injections. The gastric disturbances can be greatly minimized by the use of our new esters and by administration in enteric capsules.

Injected into the tissues, chaulmoogra oil and its derivatives are more rapidly absorbed from the muscle than from the subcutaneous tissues or the serous spaces. Moreover, when injected subcutaneously, between the muscles or into the serous spaces, there is an extensive coagulation of serous exudates forming large caseous masses that are very slowly absorbed. The relative rates of absorption from muscle and from subcutaneous tissue is shown in a typical experiment in which 0.2 cc. of the butyl ester was injected intramuscularly in two places and subcutaneously in one location on the same rabbit. Chemical analyses showed the following amounts in terms of chaulmoogric acids were absorbed from each locality.

TABLE 1.

Comparison of Intramuscular and Subcutaneous Absorption

1.	Intramuscular	118	mgs. absorbed per day
2.	Intramuscular	108	" " " "
3.	Subcutaneous	66	" " " "

Since intramuscular injections of chaulmoogra oil and its derivatives have been practiced so extensively in the treatment of leprosy, absorption from the muscle tissue has been especially investigated. The primary purpose of the Heiser and other mixtures of chaulmoogra oil with lighter oils was to secure more rapid absorption of the heavy chaulmoogra oil, which solidifies at 22°—23° C.; and one of the special recommendations of the ethyl esters has been that, being light fluids, they should be more rapidly absorbed than the oil or its mixtures. Chemical analyses do not wholly support these expectations. While our analyses do show some variation in the rates of absorption of the oil and its derivatives, the difference is not so great as expected. The relative rate of absorption of crude chaulmoogra oil and some of its derivatives, when injected intramuscularly in 0.2 cc. doses into the same rabbit, is shown in the following table.

TABLE 2

Comparison of Intramuscular Absorption of Chaulmoogra Oil and its Derivatives

SUBSTANCE	Chaulmoogric acids content, estimated in mgm.	Amount of chaulmoogric acids absorbed per day in mgm.
Chaulmoogra oil	180	85
Heiser's mixture[1]	180	85
Methyl ester	172	90
Butyl ester	144	95
Ethyl ester	162	100
Propyl ester	154	100
Amyl ester	136	100

[1] 0.4 c.c. injected because this mixture is half inert oil.

From the results of these analyses, it is apparent that the advantages which any of these derivatives may possess over crude chaulmoogra oil for therapeutic purposes is not great from the point of view of absorption; the advantage that some of the derivatives do possess is that they are less irritating and produce less local reaction in the tissues.

The rate of absorption varies with the amount injected and is roughly proportional to the size of the dose, within the limits of 0.1 to 0.4 cc. tested, as is shown in the following experiment with the butyl ester injected intramuscularly in different doses into the same rabbit.

TABLE 3

Relation of Size of Dose to Intramuscular Absorption

DOSE IN TENTHS C C.	Estimated chaulmoogric acid content of dose in mgm.	Mgm. of chaulmoogric acids absorbed per day
0.1	72	44
0.2	144	84
0.3	216	116
0.4	288	168

The rate of absorption from the muscles of the rabbit also appears to be influenced by the size of the animal, as is shown by the following absorption tests of the butyl ester injected in the same dose into rabbits of different weights.

TABLE 4

Relation of Weight of Animal to Intramuscular Absorption

RABBIT No.	Weight in grams	Dose of butyl chaulmoogrates in C C.	Estimated chaulmoogric acid contents of dose in mgm.	Mgm. of chaulmoogric acids absorbed per day
1	1760	0.2	144	58
2	3480	0.2	144	84
3	3820	0.2	144	120

Injected directly into the circulation, chaulmoogra oil derivatives are removed very rapidly from the blood. An animal dying immediately from a lethal intravenous dose may show a small amount of chaulmoogric acids in the blood; but an animal killed 15 minutes after a sublethal dose shows no trace of them. This method of administration undoubtedly secures the most rapid absorption of the drug, but the chaulmoogrates are relatively toxic by this route and only small doses can be given.

Our investigation, so far as it has gone, indicates that the fate of the absorbed chaulmoogric acids, in whatever compound or way they may be introduced into the animal body, is the same; that is, they behave like other fats taken into the body and their fate is simply a question of fat metabolism.

In ordinary therapeutic doses chaulmoogra oil or its derivatives appear to be rapidly oxidized in the body metabolism. The evidence of this is of two kinds: First, it has not been possible to detect chaulmoogric acids in any fluids or tissue of the rabbit, even after long continued administration of therapeutic doses; second, in order to determine this oxidization more exactly, mice have been injected intraperitoneally with relatively large doses of chaulmoogra oil and of the various derivatives, and after a few days the whole mouse has been analysed for chaulmoogric acids. These experiments have shown that a considerable proportion of the chaulmoogric acids injected have been oxidized, at least to the extent that they are no longer optically active, in the bodies of the mice. The results of one such experiment are shown in table 5.

If, however, very large doses of chaulmoogra oil or its derivatives be administered at frequent intervals until a large amount has been given, chaulmoogric acids may be found in the blood, liver and fat. Thus 2 cc. of the butyl esters were injected intraperitoneally daily into a rabbit weighing two kilos, until 40 cc. had been given. On analysis of the blood and organs, 1,000 mgm. of chaulmoogric acids were found in the liver, but none elsewhere. To another rabbit, 4 cc. of the chaulmoogric acids were given daily by stomach tube until 40 cc. had been administered.

TABLE 5

The Rate of Oxidation of Chaulmoogra Oil and its Derivatives in the Mouse

SUBSTANCE	Amount injected in C C.	Chaulmoogric acid content estimated in mgm.	Mgm. of chaulmoogric acids recovered after 48 hours	Mgm. of chaulmoogric acids oxidized in 48 hours	Percentage of chaulmoogric acids oxidized in 48 hours
Chaulmoogra oil.....	0.1	90	54	36	40
Butyl esters.........	0.1	72	32	40	55
Methyl esters........	0.1	86	30	56	65
Ethyl esters.........	0.1	81	24	57	79
Propyl esters........	0.1	77	12	65	84
Amyl esters	0.1	68	4	64	94
Benzyl esters........	0.1	68	0	68	100

Analyses showed 50 mgm. in the blood, 140 mgm. in the liver and 280 mgm. in the stored fat.

Fractionation by appropriate chemical methods of the chaulmoogrates found in the blood and tissues of animals treated with large doses, into fatty acid, fat and phospholipoid fractions, has demonstrated that the chaulmoogric acids may be present sometimes free, sometimes combined with glycerol as fat, sometimes in both forms and, apparently, at times in part as the phospholipoid, lecethin.

From the results of somewhat extended experiments along the above lines, we can construct a tentative scheme of the fate of chaulmoogra oil and its derivatives in the animal body. In ordinary therapeutic doses the chaulmoogric acids or their compounds are, after absorption, distributed by the blood to all of the tissues, which take them up as their physiologic needs for lipoids demand and rapidly oxidize them in their metabolism. There is no accumulation or storage in any tissue or organ, even after long continued treatment. On the other hand, if very large doses be administered at frequent intervals over a considerable period of time, there results, as in the case of an excess intake of any fats, a chaulmoogric lipaemia; the chaulmoogric acids are taken up by the organ chiefly concerned in fat metabolism, the liver, and the excess is stored in the adipose tissue as fat. In whatever compound and by whatever route the chaulmoogric acids are administered, they are probably hydrolized to the free fatty acids and then recombined to a greater or less extent with glycerol to form fat. If our findings of a small amount of chaulmoogric acids in the lecethin fraction be substantiated by further investigation, then it would appear that the chaulmoogric acids are able to take the place of other fatty acids in the synthesis of lecethin and possibly other phospholipoids.

The final step in our biochemical investigations, and one of particular importance from the point of view of the therapy of tuberculosis, is that of the penetration of the tubercle by the chaulmoogrates. So far as this part of the investigation has proceeded, the results have been completely negative; the chaulmoogric acids do not appear to be capable of penetrating the tubercle in quantities that can be recognized by our tests. This conclusion is based not only upon analyses of lung and joint tubercles after extended therapeutic treatment, but also after repeated large local injections about living joint tubercles and after immersion of joint and lung tubercles, removed at necropsy, in solutions of sodium chaulmoogrates for days at incubator temperature. These negative results conform with the observations of Wells[1] and his collaborators in their experimental study of the penetration of various substances into the tubercle, namely, that, while the tubercle is readily permeable to crystalloids, it is scarcely, if at all, permeable to colloids.

III. THE THERAPEUTIC ACTION OF CHAULMOOGRA DERIVATIVES IN LEPROSY AND EXPERIMENTAL TUBERCULOSIS

Chaulmoogra oil and its derivatives are definitely toxic; but, except for certain esters of which the toxic action is due largely to the alkyl rather than to chaulmoogric acid radical and unless introduced directly into the circulation, this toxicity is not great. Administered by mouth, the primary effect is nausea. To rabbits, which appear to be incapable of vomiting, 5 cc. of chaulmoogric acids or of the esters can be given by stomach tube, or the same amount of the esters by intraperitoneal injection without apparent ill effect; but, if this relatively enormous dose be repeated daily, there is progressive loss of weight and finally death of the experimental animal. However, a total of 40 to 50 cc. in daily 5 cc. doses has been given to rabbits weighing 2.5 to 3 kilos. In one case, 10 cc. of the butyl ester was given by stomach tube to a 2.6 kilo rabbit on three successive days, when the animal was found to be so ill that it was sacrificed for chemical examination.

Injected directly into the circulating blood chaulmoogra derivatives are much more toxic. This is probably due in part to the rapid absorption of the drug, but also in case of the esters and their emulsions to the mechanical action of pulmonary emboli. These emboli are produced not only by the oily droplets of the esters, but also by a coagulation precipitate in the blood plasma, similar in nature to that produced in serous exudates after subcutaneous and intraperitoneal injections. Twelve cc. of a 3 per cent. solution of sodium chaulmoogrates injected intravenously into a 4 kilo rabbit in a period of 12 minutes was immediately fatal; but 10 cc. of the same solution injected intravenously into a 3.8 kilo rabbit in a period of 15 minutes produced no apparent ill effect on the experimental animal. One cc. of the propyl ester injected intravenously into a 2.5 kilo rabbit was immediately fatal; but a single dose of 0.5 cc. is tolerated. If, however, the smaller dose be repeated daily, the animal succumbs after several days to the cumulative action. These experimental data on the relatively high toxicity of the esters and their emulsions injected intravenously are of importance because this method of treatment is now being practiced in leprosy. It is evident that great caution should be exercised in the dosage and the interval between doses. Rabbits treated intravenously with therapeutic doses of the esters or their emulsions show marked hypertrophy of the lungs.

The standard adopted for calculating the dosage for treatment of experimental animals has been an equivalent per body weight of the maximum dose commonly used in the treatment of lepers, namely, 5 cc. of a 3 per cent. solution of the sodium salts intravenously and 5 cc. of the esters intramuscularly. This dosage, calculated on the basis of a 70 kilo man, is equivalent to 0.0715 cc. per kilo of body weight. This standard dose has been modified to suit experimental conditions, using fractions or multiples of it up to the largest tolerated dose. In case of the ethyl esters, the standard dose of 0.0715 cc. per kilo of body weight has been found to be the largest dose that can be used continuously, because of severe local reactions from larger doses. The butyl and propyl esters can be administered in much larger doses without severe reactions. We have been especially interested in intravenous injections of emulsions of the esters, since chemical analyses have shown that by this method of administration of tolerated doses it is possible to maintain constantly in the lungs a concentration of the chaulmoogric acids far above that proved to be bactericidal for tubercle bacilli in the test tube. While this concentration of the chaulmoogrates is probably largely in the capillaries in the form of emboli, it should afford most favorable conditions for absorption by the pulmonary tissues and penetration of pulmonary tubercular lesions.

With the cooperation of Doctors Morrow and Miller of the Dermatological Department of the University of California Medical School, we have had the opportunity to study the therapeutic action of the esters of chaulmoogric acids on a limited number of lepers at the San Francisco Isolation Hospital. This personal experience in the treatment of leprosy has been of great value in establishing a standard of therapeutic action of the chaulmoogrates, with which to compare the results in experimental tuberculosis. From our limited experience with leprosy, we are of the opinion that the therapeutic action of the esters of chaulmoogric acids is feeble. Early cases do improve under this treatment; but more advanced cases have shown little or no clinical or bacteriological change in their condition after more than a year of treatment. Chaulmoogra oil and its derivatives are the only drugs that have any definite therapeutic action in leprosy and their use in this disease is therefore amply justified; but the chaulmoogrates are nevertheless far from being ideal therapeutic agents.

Our therapeutic experiments on tuberculosis in rabbits have been in progress nearly a year and are not yet completed. Several hundred rabbits have been employed in these experiments; and these animals have been treated with the different derivatives that we have prepared in varying dosage and by different methods of administration. The therapeutic results, if any, are not striking enough to permit definite conclusions being drawn until all of these experiments have been terminated and the results carefully checked up. While deferring final judgment until all of the data are in hand, the general impression from the uncompleted experiments is that the therapeutic action of the derivatives of chaulmoogra oil on experimental tuberculosis is slight, if any.

This impression is supported by the results of our biochemical investigations and by experiments being conducted to determine the bactericidal activity of the chaulmoogrates *in vivo*. Our biochemical studies have shown that it is not possible to obtain an appreciable concentration of the chaulmoogric acids in the circulation by the administration of therapeutic doses, and that it has not been possible to secure their penetration into the tubercle. In an attempt to study the bactericidal action of the chaulmoogrates *in vivo,* guinea pigs have been inoculated from the tubercular lesions of rabbits of our therapeutic series that had been treated with the various derivatives, administered by different routes, in different dosage, for variable lengths of time. In no case have the guinea pigs failed to become infected. Pursuing this line of experimentation further, injections of sodium chaulmoogrates or esters have been made directly into circumscribed caseous joint tubercles of living rabbits, in doses five to ten times the bactericidal concentration *in vitro*. After variable lengths of time, the contents of these injected tubercles have been removed and inoculated into guinea pigs. In every case, the guinea pigs have developed tuberculosis. These experiments demonstrate that the bactericidal activity of the chaulmoogrates on tubercle bacilli *in vivo* is decidedly less than *in vitro*. Whether this is due to a failure of the drug to diffuse into all parts of the tubercle, or to a fixation or oxidation of the chaulmoogric acids by the tubercle is undetermined. In any event, the failure of the chaulmoogrates to penetrate the tubercle and their enfeebled bactericidal activity *in vivo* indicates that the most we can expect therapeutically is some inhibition of the tuberculous process and not a radical cure. The complete data of this investigation, including that of the incompleted therapeutic experiments, will be published later.

In conclusion, I should like to express to the Research Committee and to the National Tuberculosis Association the appreciation that I have for their interest and financial assistance in this work, and above all I should like to express my approval of the policy of the Association to support research along lines relating to tuberculosis. As Dr. Lewis has so well said, all progress in the control of tuberculosis must depend upon accurate knowledge of the disease in all its relations, and such accurate knowledge can only be obtained through carefully conducted research.

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No. XVI

Reprinted from The American Journal of Tropical Medicine
Vol. II, No. 4, July, 1922

CONTRIBUTIONS TO THE BACTERIOLOGY OF LEPROSY[1]

I. THE DIPHTHEROID IN LEPROSY

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The presumptive causal organism of leprosy, the acid-fast *Bacillus leprae*, was discovered in leprous lesions by Hansen in 1871. Although subsequently numerous attempts have been made to cultivate this organism and repeated claims of success have been made, bacteriologists are at the present time extremely skeptical that *Bacillus leprae* has ever been grown on artificial media. If this skepticism be justified, no other bacterial organism has so long resisted all attempts at artificial cultivation.

Nevertheless, competent bacteriologists have cultivated repeatedly certain more or less acid-fast bacteria from the lesions and organs of lepers. If we exclude organisms that have been encountered only occasionally in the cultures, of which many are obviously contaminations, the bacteria that have been cultivated more or less constantly from lepers are of two types, namely: First, partly acid-fast diphtheroids, first cultivated by Bordoni-Uffreduzzi (1), in 1887; and second, chromogenic acid-fast bacilli, first cultivated by Clegg (2), in 1909, in symbiosis with amoebae and cholera vibrios. This paper is concerned exclusively with the partly acid-fast diphtheroid organisms.

The literature records the following cultivations of these partly acid-fast diphtheroids from the lesions and organs of lepers: Bordoni-Uffreduzzi (1), in 1887, from the bone marrow of a leper, on glycerinated peptone blood serum; Babes (3), in 1889, from various organs of twelve lepers, on glycerinated blood serum and plain agar; Gianturco (4), in 1889, from a non-ulcerating cutaneous nodule of a leper, on glycerine agar; E. Levy (5), in 1897, from a non-ulcerating leprous nodule, on glycerine agar streaked with human blood; Spronck (6), in 1898, from a skin tubercle and from the bone marrow of two cases of leprosy, on neutral glycerinated potato medium; Czaplewsky (7), in 1898, from nasal secretions and discharges from a throat ulcer of a case of leprosy, on inspissated glycerinated sheep's blood serum; Shibayama (8), in 1899; Teich (9), in 1899, from nasal secretions, incised and excised nodules of five cases of leprosy, on glycerine agar streaked with human blood; Baranikow (10) in 1899, from early nodules of two cases of leprosy, on media made from human brain and from transudates; Kedrowski (11), in 1901, from incised follicles and excised nodules of three cases of leprosy, on placental extract agar; F. Levy (12), in 1902, four times in glycerine veal broth and on slabs of red beet; Klitin (13), in 1905, from excised nodules of four cases of leprosy, on various media; Bayon (14), in 1911, from an excised nodule of a leper, on placental extract agar and on horse serum maltose agar to which was added ground up smegma bacilli; Williams (15), in 1911, from non-ulcerating nodules of an unstated number of lepers in Persia and India, on "lemco" glucose agar; Ophüls (quoted by Wolbach and Honeij (16); and Wolbach and Honeij (17), in 1914, from an excised epitrochlear lymph gland of a case of leprosy, on ascitic fluid dextrose agar.

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[1] Read at the eighteenth annual meeting of the American Society of Tropical Medicine, May 2 and 3, 1922, Washington, D. C.

The literature records that, from 1887 to 1914, at least 16 bacteriologists cultivated partly acid-fast diphtheroids 46 + times from various tissues and organs of 40 + cases of leprosy in different parts of the world. How many others have cultivated such organisms and discarded them as secondary invaders or as contaminations, because they did not conform completely in morphology and staining with *Bacillus leprae* in the tissues, will probably never be known. Since 1914, owing to the doubts that have arisen over the identity of these cultural organisms, interest has declined and the literature contains no further references to attempted cultivations of *Bacillus leprae*.

It is admittedly difficult to identify with certainty bacteria cultivated by the earlier bacteriologists, owing to imperfect descriptions of these organisms; but the diphtheroid cultivable from leprous lesions possesses cultural, morphological and tinctorial peculiarities which are so striking and characteristic that they can not readily be overlooked or confused with those of other diphtheroids. These characteristics are briefly: Gray colonies with an elevated, whitish center and a serrated border from which secondary colonies may arise; large size and extreme pleomorphism of the bacilli; the formation of arthrospore-like bodies; and acid-fast staining of granules, segments or whole organisms. An examination of the descriptions by the authors quoted, made in the light of these peculiar characteristics and of my own experience with cultivations from leprous lesions, make it reasonably certain that most, if not all, of them have isolated one and the same diphtheroid organism from leprosy.

The first purpose of my investigation was to substantitate, if possible, the more or less constant cultivability of a pleomorphic, partly acid-fast diphtheroid from leprous lesions. The material for this investigation was obtained from lepers confined in the San Francisco Isolation Hospital. These lepers were under treatment with intramuscular injections of esters of chaulmoogric acids. As difficulty was constantly experienced in persuading these patients to continue the intramuscular injections, it was not considered advisable to further antagonize them by repeated biopsies. Consequently, the material used for this cultural study was obtained chiefly from nasal ulcerations and occasionally from open leprous lesions of the skin. In a few cases aseptic incision of non-ulcerating leprous nodules was performed.

The routine procedure with the contaminated leprous material, collected on sterile swabs, was to add a little sterile saline to the tubes, rub up the material on the swabs in the saline and plate this saline suspension in three dilutions on glycerinated veal broth agar. These plate cultures were incubated at 37°C. With experience, it was possible to identify with a hand lens the colonies of bacteria ordinarily developing in these plate cultures. The suspected diphtheroid colonies were examined in stained smears unter the microscope and the diphtheroid colonies transplanted on glycerine agar slants for further study. The material taken aseptically from non-ulcerating nodules was inoculated directly on slants of various media.

In 312 cultivations from the nose and open skin lesions of 16 lepers, four types of diphtheroids have been encountered, which can be distinguished from one another by colony characters, carbohydrate fermentations, morphology and staining peculiarities. The distribution of these four types in the last 164 cultivations, with reference to the number of cultivations from each patient, the type and progress of the disease and the presence or absence of acid-fast *Bacillus leprae* in the nasal secretions, is shown in table 1. The first 148 cultivations are not included in this table, because the plate cultures were not incubated sufficiently long to secure the development of visible colonies of diphtheroid IV.

TABLE 1

Types of diphtheroids cultivated from nasal secretions of lepers

PATIENT	TYPE OF DISEASE	PROGRESS OF DISEASE	ACID-FAST BACILLI IN NASAL SECRETION	TOTAL NUMBER OF CULTIVATIONS	THEROID TYPE I ISOLATED TIMES	DIPHTHEROID TYPE II ISOLATED TIMES	DIPHTHEROID TYPE III ISOLATED TIMES	DIPHTHEROID TYPE IV ISOLATED TIMES
Abdul	Tubercular	Advanced	+	6	3	2	2	3
Anman	Tubercular	Advanced	+	20	—	12	—	3
Bemat	Tubercular	Advanced	+	15	1	7	5	3
Dan	Tubercular	Advanced	+	13	—	6	—	5
Fong	Anesthetic	Moderate	0	7	2	—	—	—
Frank	Tubercular	Moderate	±	11	2	4	—	—
Garcia	Macular, anesthetic	Early	0	3	1	—	—	—
George	Tubercular	Moderate	±	2	—	1	—	—
Kim	Mixed	Moderate	±	18	1	3	—	—
Lucy	Mixed	Moderate	±	8	5	5	—	—
Lum	Anesthetic	Moderate	0	8	3	1	—	—
Silva	Tubercular	Advanced	+	14	6	2	2	2
Tony	Tubercular	Advanced	+	18	1	7	2	10
Wong	Tubercular	Moderate	0	6	—	2	—	—
Yat	Tubercular	Moderate	±	15	1	9	1	—
Total..................................				164	26	61	12	26

The essential characters of these four types of diphtheroids are as follows. Cultural characteristics not noted are those common to the diphtheroid group.

DIPHTHEROID I

Colonies on glycerine agar well developed after forty-eight hours' incubation; small, moist, circular, elevated with a central boss; light gray and translucent. None of the carbohydrates are fermented. Bacilli rather short, of medium thickness, cylindrical or with tapering ends, occasionally slightly clubbed. Organisms stain uniformly or with a single unstained cross-bar; Gram-positive; non-acid-fast. This diphtheroid has been cultivated from the nasal secretions of lepers independently of the type or progress of the disease and of the presence or absence of *Bacillus leprae* in the secretions. The characters of this diphtheroid identify it as the well known pseudo-diphtheria bacillus of Hoffmann (18), or *Corynebacterium pseudo-diphthericum* Eberson (19), and a common habitant of the throat and nasal passages of man in health and disease.

DIPHTHEROID II

Colonies on glycerine agar well developed after forty-eight hours' incubation; thin, flat, moist, translucent, dirty gray in color. Dextrose, lactose, saccharose and maltose fermented vigorously, glycerine slightly, with the production of acid but no gas. Bacilli medium length to long, slender, cylindrical, swollen in the middle or clubbed. Organisms stain segmentally or with polar granules; Gram positive; non-acid-fast, but many of the bacilli are of a polychromatic (reddish-purple) color after staining by the acid-fast method. This diphtheroid was the one most frequently encountered in the cultures from the nose and open skin lesions of lepers. Like diphtheroid I, it was cultivated from both early and advanced cases of all types of leprosy and independently of the present of acid-fast bacilli in the secretions, although it appears to have a predilection for ulcerative lesions.

DIPHTHEROID III

Colonies on glycerine agar more rapidly growing, larger and more elevated than diphtheroid II; convex, cream to pale salmon colored. Carbohydrate fermentations are the same as diphtheroid II, except that lactose is fermented feebly. Morphology and staining similar to diphtheroid II. This diphtheroid differs but slightly from type II, except in the very distinct colony characteristics. Diphtheroid III has been cultivated from the nasal secretions of only a few cases of leprosy.

Diphtheroid II and III do not correspond to any hitherto adequately described species and are not included in Eberson's (19) classification of the diphtheroid group. Morphologically and in staining both types are indistinguishable from the diphtheria bacillus, but differ from it in colony characters, carbohydrate fermentations and pathogenicity. They are perhaps identical with some of the diphtheria-like organisms inadequately described in the recent extensive literature on the bacteriology of war wounds. I have cultivated diphtheroid II from non-leprous chronic ulcers.

DIPHTHEROID IV

Colonies on glycerine agar, recognizable in the first cultural generation only on the fourth day of incubation, or later; variable within certain limits; typically of medium size, gray, with an elevated, whitish center and an irregular serrated or lobulated border from which secondary colonies may arise. Dextrose and maltose fermented vigorously, saccharose and glycerine feebly and sometimes evanescently, with the production of acid but no gas. Bacilli extremely pleomorphic; characteristically large, but size variable; coccoid to long, occasionally streptothricoid; clubbed or irregularly swollen; segmented, often appearing as short chains of coccoid elements of variable size; some of the segments may become much swollen, stain intensely and become detached as arthrospore-like bodies. Organisms stain with ordinary aniline dyes segmentally and show metachromatic granules; Gram positive; partly and variably acid-fast, especially in the first cultural generation. Acid-fast staining is frequently restricted to certain segments, large spore-like granules or to the clubbed ends, and may be lost in subsequent transplants of the organism.

This diphtheroid has been cultivated from the nose of only advanced cases of tubercular or mixed leprosy, showing many acid-fast *Bacillus leprae* in the nasal secretions. Cultures from early cases and of the anesthetic type of leprosy, of which the nasal secretions contained no acid-fast bacilli, have been consistently negative. Moreover, patients, who had been long under treatment with the esters of chaulmoogric acids with improvement of symptoms and diminution or total disappearance of acid-fast bacilli from their nasal secretions, were either negative for diphtheroid IV or this diphtheroid could be cultivated only during the earlier stages of the treatment. In addition to the 26 isolations of this diphtheroid from the comtaminated material from the nasal secretions of lepers, it was also cultivated in pure culture once in three attempts, from material obtained by asceptic incision of leprous nodules.

Diphtheroid IV cannot be identified with any of the adequately described diphtheroids cultivated by Frankel and Much (20), Billings and Rosenow (21), Negri and Mieremet (22), Buntin and Yates (23), Hine (24), Bloomfield (25), Fox (26), Torry (27), Neubauer (28) and others in their extended studies of the bacterial flora of lymphatic glands and other tissues of man in health and diseases other than leprosy. On the other hand, this diphtheroid does possess the characteristic colonies, extreme pleomorphism and partial acid-fastness of the diphtheroid that has been cultivated repeatedly since Bordoni-Uffreduzzi (1887) to Wolbach and Honeij (1914) from leprous lesions.

Having substantiated the claims of previous investigators that a pleomorphic, partly acid-fast diphtheroid can be cultivated repeatedly from open as well as non-ulcerating leprous lesions, I have next directed my efforts to discover a possible saprophytic source of this diphtheroid, as bearing on its parasitism and etiologic relation to leprosy. Passing over the unproductive cultural investigations of normal nasal secretions, chronic ulcerations and external sources, I would direct attention to the bacteriology of smegma praeputii.

Microscopic examination of a smear preparation of smegmal secretion, stained with carbol fuchsin, decolorized with acid and counterstained with methylene blue, shows two types of microorganisms, staphylococci and bacilli. The bacilli, however, present the greatest diversity of form, including coccoids, rods of various length and thickness, diphtheroids, short filaments and branching forms, giving the impression of a very diverse bacilliform flora. This impression is further emphasized by the fact that some of the bacillary forms are acid-fast and retain the carbol fuchsin, while others are stained by the methylene blue. The acid-fast forms are predominantly the more slender rods of uniform thickness, but careful search will disclose an occasional coccoid, diphtheroid or streptothricoid form that is partly or wholly acid-fast.

If some of this smegmal secretion be cultured in proper dilutions on glycerine agar plates, it will be found that from this apparently diverse bacterial flora only colonies of staphylococci and of a diphtheroid develop on the plates. The diphtheroid colonies are slow growing, gray, with an elevated, whitish center and serrated border. The diphtheroids are large and extremely pleomorphic, much segmented, develop in old cultures arthrospore-like bodies and frequently show acid-fast granules or segments. They ferment dextrose, saccharose, maltose and glycerine.

What is the relation of this cultivable diphtheroid to the apparently diverse bacilliform flora of smegma praeputii? The most obvious conclusion might be that this diphtheroid is the only one among several species that is able to grow on glycerine agar. On the contrary, a consideration of the transitional stages in morphology and acid-fastness between the different bacillary types in smegma, the cultivability of this diphtheroid from smegmal secretions after treatment with Petroff's sodium hydroxid solution or antiformin, and the fact, that it can become completely acid-fast on a special medium, as will be discussed in a succeeding paper, leads me to believe that this diphtheroid is the cultural stage of a very pleomorphic, facultative acid-fast bacillus, the so-called *Bacillus smegmatis*.

A review of the literature on the bacteriology of smegma praeputii supports this conclusion. Czaplewski (29), Laser (30), Frankel (31) and Neufeld (32) were able to cultivate only one kind of bacillus from smegma. Although the acid-fastness of this bacillus was not lost in all cases in the first cultural generation, it quickly and almost completely disappeared when the cultures were transplanted to fresh media. Weber (33) observed that cultures of the smegma bacillus retained their acid-fastness on a culture medium containing lanolin. Gillert and Smitt (34) cultivated a diphtheroid from smegma, after antiformin treatment, which was in no case acid-fast. Hartmann (35) was able to cultivate only a pleomorphic diphtheroid from smegma praeputii treated with antiformin. This diphtheroid was not acid-fast; but, if some fatty material such as lanolin was spread over the slide and slightly warmed before making the preparation, the greater proportion of the bacilli stained acid-fast. Hartmann concludes that this pleomorphic diphtheroid, resistant to antiformin, is probably *Bacillus smegmatis;* and that the acid-fastness of this organism is not an inherent property, but is the result of the protective action against acids of the coating of fatty smegmal secretions.

Whether or not we accept the conclusion that the diphtheroid cultivable from smegma praeputii is *Bacillus smegmatis* is immaterial for my purpose, since in either event the essential fact remains that this diphtheroid from smegma possesses cultural, morphological, biochemical and tinctorial characteristics which are apparently identical with those of the diphtheroid cultivable from the lesions and organs of lepers and considered by some authors as the cultural form of *Bacillus leprae.*

SUMMARY

1. The pleomorphic, partly acid-fast diphtheroid of Bordoni-Uffreduzzi and other authors can be cultivated more or less constantly from nasal and other open lesions of lepers, as well as from non-ulcerating leprous lesions.

2. This diphtheroid differs in its large size, extreme pleomorphism, peculiar colonies, carbohydrate fermentations and

partial acid-fastness from all diphtheroids from other sources adequately described in the literature.

3. A search for the possible saprophytic source of this diphtheroid from leprous lesions has disclosed that it is apparently identical with a diphtheroid cultivable from smegma praeputii, which is probably a cultural form of the pleomorphic and facultative acid-fast *Bacillus smegmatis.*

SUMARIO

1. El difteroide pleomórfico y parcialmente ácido-resistente descríto por Bordoni-Uffreduzzi y otros autores, puede cultivarse, con más o menos constancia, de las lesiones nasales y abiertas de los leprosos. Asi mismo de lesiones sin ulcerar.

2. Este difteroide difiere de los otros difteroides de diversos orígenes ya descritos en la literatura médica, por su tamaño grande, pleomorfismo, peculiaridad en su formación de colonias, fermentación de carbohidratos y ácido-resistencia parcial.

3. Una indagación del orígen posiblemente saprofítico de este difteroide de lesiones leprosas, nos hace creer que es aparentemente idéntico con el difteroide cultivable que se obtiene del smegma preputii. Este último es probablemente una forma cultural del pleomórfico y ácido-resistente-facultativo *Bacillus smegmatis.*

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ACTION CURRENTS IN STOMACH AND INTESTINE

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Reprinted from the THE AMERICAN JOURNAL OF PHYSIOLOGY
Vol. 58, No. 3, January, 1922

ACTION CURRENTS IN STOMACH AND INTESTINE

WALTER C. ALVAREZ IN COLLABORATION WITH LUCILLE J. MAHONEY[1]

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Received for publication July 29, 1921

My first efforts to show the action currents in the bowel were made in 1913 when I was working in the Physiological Laboratory at Harvard. At that time Dr. Alexander Forbes very kindly made a few records for me with the string galvanometer, just enough to show *a*, that currents of measurable intensity can be obtained from excised segments; and *b*, that the ordinary non-polarizable electrodes used in muscle and nerve studies are not convenient for work on the bowel.

In 1917 I again attacked the problem with the help of Dr. Robert Newell. We used segments of rabbits' small intestine contracting in a moist chamber. These segments were connected to an Einthoven galvanometer through zinc-zinc sulfate moist thread electrodes. A mechanical record was obtained at the same time by arranging a lever so that its shadow would be thrown across the slit of the camera. Although some good records were obtained, it was soon plain that few of the problems before us could be answered until better electrodes could be secured. The nature of the work demands first, that they be so designed that they can be attached quickly and easily in such inaccessible places as the cardiac end of the stomach (in the intact animal); second, that such attaching and detaching be done without trauma such as that involved in sewing on threads; third that the apparatus move with the viscus without sliding over its surface; fourth, that it work perfectly in any position; fifth, that it work under Locke's solution; and sixth, that it maintain a constant potential. Incidentally, I hoped to get an electrode which would not have to be made up fresh every few days. After experimenting during the last three years with almost every type of electrode heretofore described I have at last designed one which answers all the demands made upon it. As slight modifications are still being made from time to time, I will reserve a full description of the apparatus for a subsequent paper. Suffice it to say here that it is a small calomel electrode attached to a simple clamp.

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[1] This work was made possible through the kindness of: Mr. T. E. Bibbins, Dr. W. B. Coffey, Mr. Mortimer Fleishhacker, Dr. John Gallwey, Mr. E. Clemens Horst, Dr. N. W. Jones, Mr. G. A. Mattern, Dr. Langley Porter, Dr. H. A. L. Ryfkogel and Dr. A. J. Zobel, who paid Miss Mahoney's salary during the past year.

I wish to thank Dr. Elizabeth Schultze for her help in the work with the Einthoven galvanometer; Mr. L. B. Clark for his help in the construction of apparatus, and Mr. Frank Rieber for the loan of galvanometers.

Another problem at first was to get good contractions in the bowel without having it immersed in Locke's solution. The trouble is that the oxygenated bath which is so essential to the normal behavior, particularly of the excised gut, tends to short-circuit a considerable part of the action current which one hopes to detect with the galvanometer. Dittler (1), in his experiments on the crop of the sea-slug Aplysia, was able to avoid some of this shorting by lowering the conductivity of the solution. He got good results with an artificial sea water in which most of the NaCl was replaced with sufficient dextrose to supply the desired osmotic pressure. My own experiments with such a solution were not promising, but at that time I was using moist thread electrodes. With the new electrodes we have gotten good records even with the segments in Locke's solution. The excised bowel will contract rhythmically for some time in a warm moist chamber, but the conditions are abnormal; the amplitude of contraction rapidly diminishes; the rate becomes slow and irregular, and the action currents not infrequently become too weak to record (see fig. 1). I spent several months working with such segments in moist chambers without getting any really satisfactory results. Some of my difficulties were due to the fact that I was using rabbits, the intestines of which have thin walls, with little muscle and, naturally, weak currents. Better results were obtained when I began using segments from cats and dogs. As will be seen later, the best results can probably be gotten by using the intact bowel, with its circulation maintained.

Another difficulty with the gastro-intestinal action-currents is that while some of them are quite large, they tend to neutralize one another on account of the lack of synchronism in the muscular activity, i.e., one segment is relaxing while another is contracting. If the digestive tract as a unit were to pulsate regularly, or if a diastaltic wave were to shoot at short intervals from the cardia to the anus, the mass of muscle involved would be so large, compared with that in the heart, that the record obtained by leading off from an arm and leg might be more of an electro-enterogram than an electro-cardiogram. As there is actually little synchronism of the movements in the various segments of the gut, we cannot expect to get good galvanometer records unless we place the electrodes close together on the viscus itself.

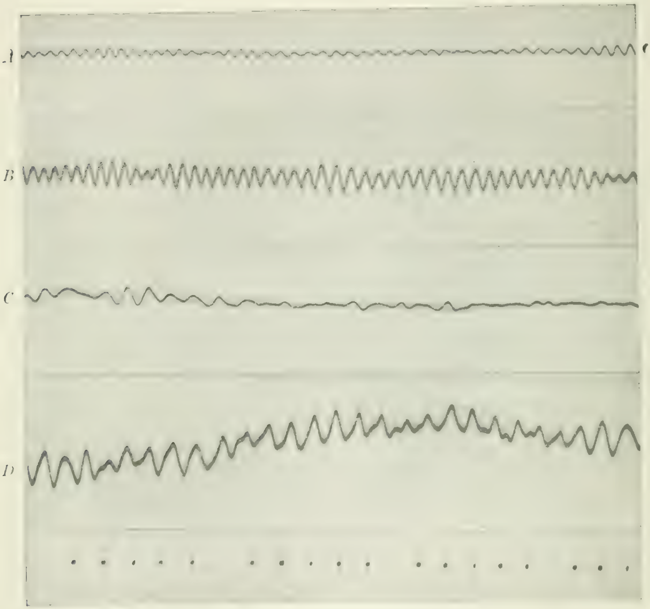

Fig. 1. Action currents from the excised duodenum, *A*, and jejunum, *B*, of the rabbit, with the gut in a bath of warm oxygenated Locke's solution (*A* and *B*); and out of the bath (*C* and *D*). The time record represents 10-second intervals with a double gap every minute.

Still another way in which I made things harder for myself at first was by using an Einthoven galvanometer. A good D'Arsonval instrument is more sensitive and better adapted for the recording of the slowly changing currents produced by smooth muscle. The disadvantage of the D'Arsonval is that the higher sensitivities are purchased at the price of increasing the weight of the moving coil, and lengthening the period of its swing. I am now using two Leeds and Northrup instruments, one with a sensitivity such that 1 mm. deflection at a meter distance equals 3×10^{-10} amp., the other with a sensitivity of 6×10^{-10} amp. The first one has a period of 7.4 seconds, the second 5 seconds. The sensitivity is greater than necessary, and I have actually had to put in shunts at times to get rid of some of it, particularly when working on the antrum of the stomach. Some experiments done with an even more sensitive D'Arsonval instrument (5.37×10^{-11} amp.) show that the best galvanometer is probably the one just delicate enough to record the currents. Greater sensitivities bring with them all sorts of difficulties from stray currents, polarizations, differences in capacity, temperature, etc. The difference in sensitivity between an Einthoven and a good D'Arsonval galvanometer can be seen in the fact that in the standard electrocardiogram 1 cm. deflection represents 1 mv. while with the better of my two instruments 220 cm. represent 1 mv. when the camera is placed at a distance of 1 meter. We intend to use the Einthoven galvanometer hereafter only when the nature of the experiment requires the elimination of all deformity and time lag from the records.

During the last few months we have been getting very satisfactory records from the stomachs and intestines of dogs, cats and rabbits opened under ether or urethane anesthesia. The best incision to expose the stomach is a transverse one, running along the lower edge of the ribs from one side to the other. The cut is made between two clamps and the skin and peritoneum are then sewed together to stop hemorrhage. The rabbits show better peristalsis if the cord is destroyed from the lumbar to the midscapular region, but the dogs and cats do not seem to need such pithing. After applying the electrodes, the wound is closed as far as possible and moist gauze is used to protect the viscera from drying. In some experiments, and especially when working near the diaphragm, we have packed off with rubber dam so as to get rid of stray currents. The animals are kept warm on an electric pad. We hope later to study the action currents of the intact animal immersed in Locke's solution, when we can get a properly enameled tank. A metal tank with the usual thermo-regulator will not do on account of the large currents which are formed in the bath. The dogs have shown the most active gastric peristalsis and are probably most suitable for work on the stomach. Rabbits do very well for studies on the intestine.

Records are made by directing the beam of light reflected from the galvanometer mirrors through the slit of a camera on to a revolving drum covered with bromide paper. In order to save space on the paper this camera is placed only 25 cm. from the mirrors. The time record is obtained by fastening a little mirror to the armature of a signal magnet so devised that the mirror will tilt when a current is sent in by way of the clock. The mechanical records are obtained by fastening a tiny mirror to one end of a straw about an inch long. The plane of the mirror is perpendicular to the long axis of the straw. The other end of the straw is fastened to the central point of the rubber membrane of an ordinary tambour. The straw passes through a hole in a little piece of stiff paper which is fastened to the edge of the tambour and serves as a fulcrum. When the membrane bulges, the mirror is tipped and the light spot moves on the drum. This tambour is connected to one of my enterographs, a simple little bellows which is fastened to the bowel by two tiny wire serrefines (6). As the whole apparatus is developing and changing from day to day as new needs arise and new knowledge is gained, further details will be given in a later paper. My object at present is simply to report progress and to point to a few things which are already clear.

Polarity of the bowel. It has been known for many years that there are gradients of electrical potential along the axes of plants and animals. The rapidly growing tip of a plant is negative to the trunk (2); the head of a hydroid polyp is negative to its stem, and anterior levels are negative to posterior ones (3). In recent years Child (4) and his students have found such electrical gradients in all the axiate animals and plants which they have studied. These gradients seem to be closely associated with, and due to, parallel ones of chemical activity or metabolism. As Miss Starkweather and I (5) have shown that there is a gradient of chemical activity in the bowel from duodenum to colon, I was much interested to see if a gradation in electrical potential could be demonstrated. Thus far considerable evidence has been obtained for such a view, but unfortunately much of the work done has not been entirely conclusive on account of the technical difficulties encountered. The muscle is in contact with the mucous membrane and this sometimes incloses masses of digesting food. Fortunately for us, the metabolic gradient in the mucous membrane seems to run quite parallel to that in the muscle. Otherwise the problem would be even more difficult. The chemical processes going on in the food seem to exert a big influence because in the rabbit the full cecum and stomach are generally markedly negative to all other parts of the tract. Another difficulty arises in the fact that the activity of the muscle varies from time to time in various parts. Moreover, in order to compare the potential of the duodenum and cecum the bowel cannot remain in the body with the various coils in contact one with another, but it must be removed and so arranged that there are no short-circuits. That means trauma, a certain amount of drying, varying degrees of asphyxia, etc. Recently we have begun to get good results with freshly removed dogs' intestines stretched out in a long bath of warm Locke's solution. In some of these experiments there appeared a definite gradient of potential from the relatively negative duodenum to a point about a foot above the ileo-cecal sphincter. From that point the gradient rose again to the sphincter as we would expect from previous work on the rhythmic gradient (6). Similar gradients have been found in some rabbits, but so far we feel that the results are only suggestive. Further work will be reported later.

The character of the action currents. As will be seen from the records, the curves obtained in different regions with the galvanometer are remarkably similar to those traced by heart levers attached to bits of muscle from corresponding parts of the tract. Naturally, the records are simplified if the electrodes are kept close together on the gut. They are placed generally within a centimeter or two of each other so that the curves will not have too many components. The most interesting thing is that the mechanical and the electrical curves correspond not only as regards rate of contraction but also as regards amplitude and form. For instance, a glance at the illustrations in my fourth paper (7) will show that the contractions of strips of muscle removed from the lesser curvature of the stomach near the cardia are small and even and that their rate is fairly rapid. Figure 2*A* shows how closely the electrogastrogram from that region corresponds to the mechanogram. On the greater curvature the amplitude of contraction is larger, the waves uneven and the rate slower—the electrogram corresponds closely (fig. 2, *B* and *D*). In strips removed from the pyloric antrum the amplitude is very large, the peaks are sharp and symmetrical and the rate is slow, corresponding to that of the intact stomach. Again, the electrogram corresponds exactly (fig. 2, *C* and *F*). In the duodenum, the mechanogram shows small, even, and rapidly recurring waves—the electrogram, has the same characteristics (figs. 3 and 4). The rate of rhythmic contraction varies inversely as the distance from the pylorus to the lower ileum. This rate in the intact animal is from 22 to 17 at the pylorus, to from 11 to 7 near the ileocecal sphincter. The rates obtained electrically are the same. The contractions obtained from segments of colon are generally slow and irregular and, again, the action currents resemble them (fig. 9 *B*). This work brings, therefore, ample confirmation of that done before with mechanical records. It demonstrates clearly again the rhythmic gradient, and the marked differences in the types of activity shown by the muscle in the different parts of the tract.

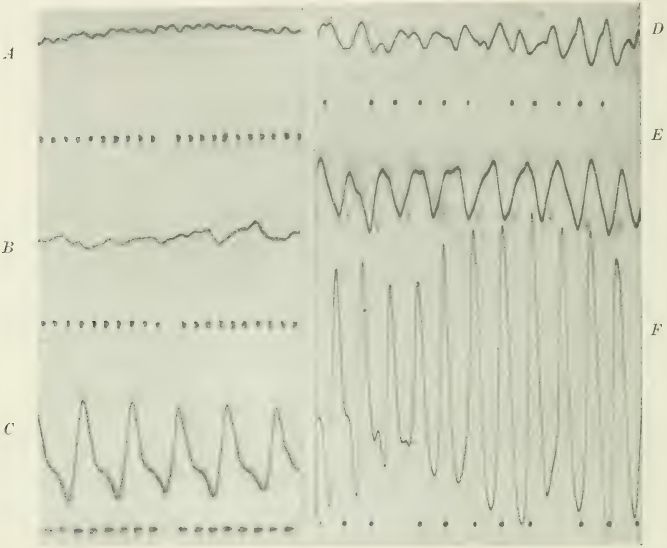

Fig. 2. To show action currents from different parts of the stomach: *A*, from the cardia of the cat; *B*, from the fundus of a cat; *C*, from the pyloric autrum of a cat; *D*, from the fundus of a dog; *E*, from the preantrum, and *F*, pyloric antrum of a dog. An upward deflection indicates negativity of the orad electrode.

The most remarkable thing about these action currents is that they are produced constantly in stomachs and intestines which, so far as the eye can detect, are absolutely motionless. Although, as we would expect, they are more powerful when the muscle is actively contracting, they are often just about as strong when no motion can be detected. Anyone who has done much work on the intestine with the abdomen open will realize what it means to have a technic which will enable him to go right ahead with his experiment without waiting, perhaps hours, for the appearance of normal peristalsis. As Auer particularly pointed out, when the abdomen is opened a hush seems to fall upon its contents. Stimuli coming through the splanchnic nerves cause an inhibition of the intestinal movements. To obviate that, it is customary to cut the splanchnic nerves or to destroy the lower part of the spinal cord.

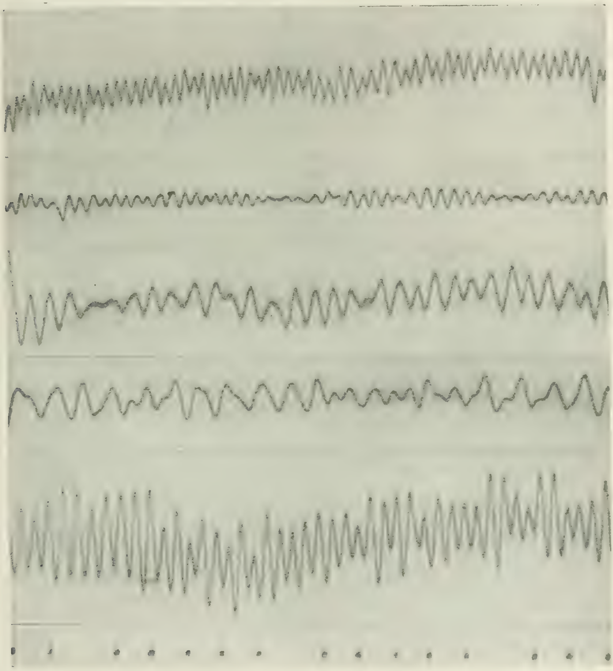

Fig. 3. To show the action currents and the rhythmic gradient in the rabbit's intestine from duodenum to ileum. The rates per minute from above downwards are: duodenum, 21.5; jejunum, 18.5; middle, 10.3; lower ileum, 7; terminal ileum, 15. The time record indicates 10-second intervals with a double gap every minute. Note irregularities due to the harmonic synthesis of components with slightly different rates. The "tone" changes in the terminal ileum come probably from the colon.

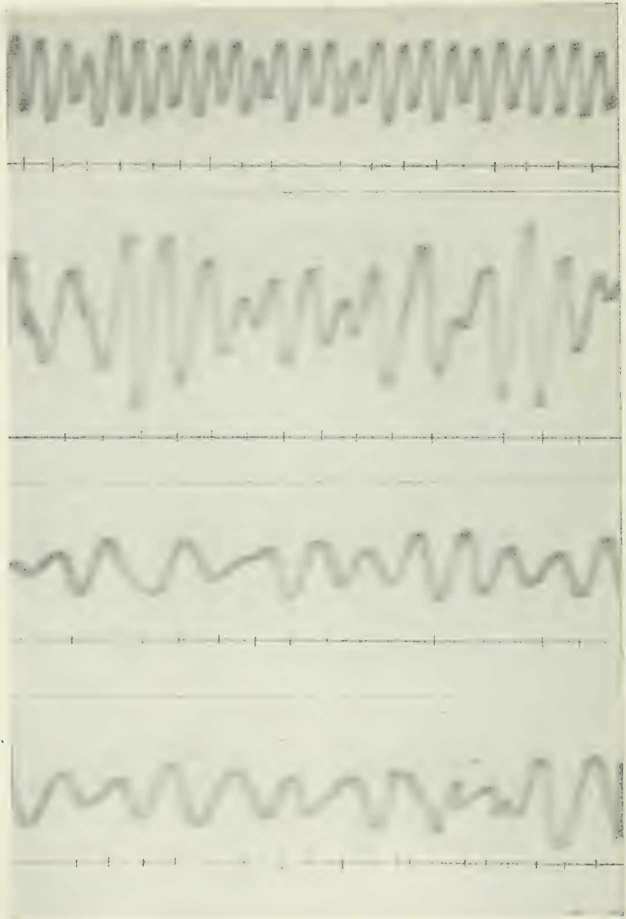

Fig. 4. To show the action currents and rhythmic gradient in the cat's intestine. The rates per minute from above downwards are: duodenum, 15.5; jejunum, 11; middle, 9; terminal ileum, 7. The time record indicates 5-second intervals with a double gap each minute.

Unfortunately, that does not always bring about an immediate return of peristalsis; and a large proportion of the animals so treated will not prove entirely satisfactory for experimental work. I have never been able to determine just why one animal will show good gastric peristalsis and ten others will not. Some workers get better results by giving castor oil, aloin or other drugs, but I have always hesitated to use such drugs before attempting "physiologic" experiments. The use of the ether or urethane is bad enough. Not infrequently, good peristalsis can be obtained only toward the end of a long experiment. After working for several years with such handicaps, it is a great pleasure to have a method which enables me to get good records as soon as the abdomen is opened. So far there have been no failures although, naturally, some animals produce more powerful currents than others.

The persistence of strong action currents in the presence of a quiet inhibited gut brings up some interesting problems. It would appear that the splanchnic nerves can block the mechanical contractions while, at the same time, they leave the rhythmic chemical processes which presumably give rise to the mechanical ones. It makes one think of an automobile with the engine running and the clutch out. From time to time some mechanism lets the "clutch" in, and motion appears. Stübel (8) and Marimon (9) have commented on this phenomenon. Stübel noticed in chickens that he could get good currents from absolutely quiet crops. Similar observations have been made also with the heart, where certain drugs or the absence of calcium will stop the contractions, but leave the action currents. Mines (10) believes that calcium helps in some way to convert the rhythmic chemical changes into mechanical contractions. The intestine offers a nice field for the study of this phenomenon. So far we have been able to show that vagus stimulation will produce temporary inhibition of the action currents followed by an increase in their amplitude. That response corresponds to the one observed at times in the mechanical record. Not infrequently faradic stimulation of the vagus strong enough markedly to affect the heart and lungs will have absolutely no effect on the electro-enterogram.

Problems in the stomach. An interesting problem has arisen in regard to the pacemaker of the stomach. As is well known, there are three waves of contraction to the minute in the stomach of man, and from two to five per minute in the laboratory animals. These waves appear, to the naked eye, to arise ordinarily about the middle of the stomach, but careful x-ray or mechanical records show that many of them develop in ripples which come from the neighborhood of the cardia. As I was able to show that strips of muscle removed from the lesser curvature next to the cardia ordinarily have a faster rate of contraction than that of any other part of the stomach, I felt that that region is probably a sort of pace-maker. In the excised bits of muscle there is more or less gradation in rate from the cardia to the pylorus. At the cardia it is about 12 per minute; in the antrum it is about 3 per minute. The next question is: why should there be a discrepancy between the rates of the excised muscle and the intact organ? In the intestine there is practically no such difference. Why should a muscle which tends to beat 12 times per minute send out over the stomach only 3 waves per minute? The best answer seemed to be that the muscle in situ beats at a slower rate because that allows it more time for recovery and insures a higher efficiency. Now, to my surprise, I find that the cardiac muscle *in situ* gives signs of contracting rhythmically 8 or 12 times per minute, as will be seen in figure 5. In some rabbits the rate is around 20 per minute. The antrum, a few centimeters away, shows potential changes from 2 to 5 times a minute, and it can easily be shown that these correspond to the visible waves coursing over the stomach. If the rhythmic action currents detected near the cardia mean that the muscle there is pulsating 12 times a minute, the next question is, what relation do these pulsations bear to the waves which course over the stomach? Do the large waves originate in one of the small ones and if so, how does that particular one happen to gain the ascendancy? It would seem that with two galvanometers working together the matter could easily be settled, but many difficulties have arisen in the interpretation of the record. In many of them there is no difference in size in the cardiac oscillations and no sign of a rhythmic tonus change. Furthermore, the cardiac rate does not seem always to be an even or a constant multiple of the antral rate. In other records it seems quite certain that the waves coursing over the stomach are arising in tonus changes at the cardia; in still others it is hard to say whether or not the tonus waves are intrinsic and due simply to a spreading of the current from the antrum. We can see that there is some spreading because the cardiac records sometimes show respiratory oscillations, and the records from the pyloric antrum nearly always show oscillations corresponding to the duodenal contractions. It is possible, of course, that the cardiac region does actually pulsate at a rate faster than that of the antral end of the stomach. Basslinger, years ago, described such a pulsation; Palugyay has observed with the x-ray small waves running

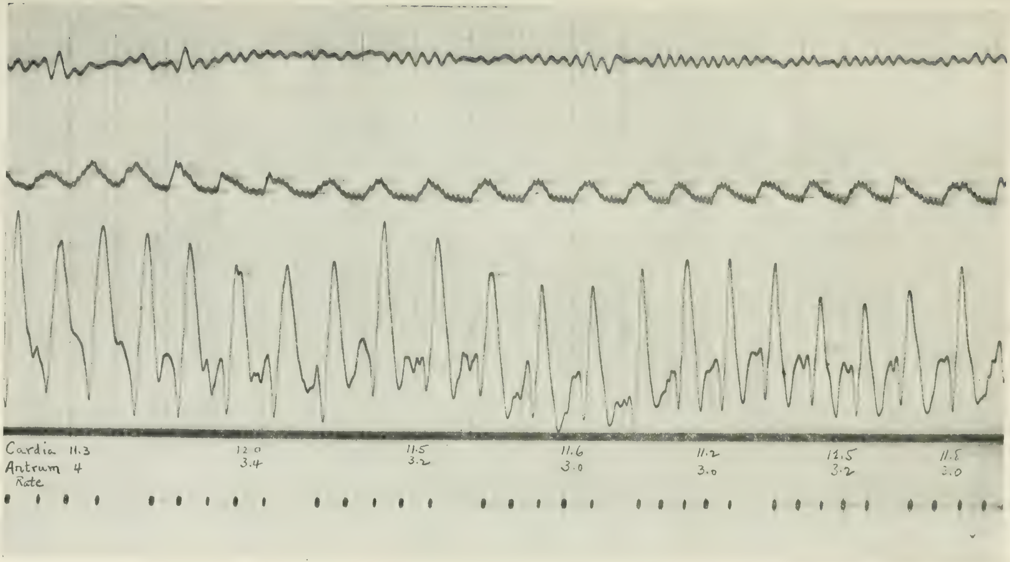

Fig. 5. From above downwards the records are: electrogram from the lesser curvature next to the cardia; mechanogram at the upper end of the pyloric antrum; electrogram from the pyloric antrum; signal magnet; time magnet at 10-second intervals. The animal was a large female dog with a poor intestinal gradient and a tendency to reverse peristalsis in the stomach. In this and all subsequent illustrations an upward deflection means that the cephalad electrode has become negative to the caudad one. The rate at the cardia varied between 11.2 and 12.5 per minute; at the antrum between 3 and 4 per minute. The smaller waves at the antrum have a rate between 19 and 24 per minute and may come from the duodenum. It will be noted that there is no constant relation between the rate at the cardia and that in the antrum. The smaller waves on the mechanogram are respiratory in origin; the large ones represent gastric peristalsis.

out from the cardia on to the fundus; waves different from those in the lower part of the stomach; and we have seen signs of such small frequent waves on some of our mechanical records. The fact that the frequent action currents from the cardia are detected also in excised stomachs shows that they are intrinsic and not arising in the bowel or adjacent organs.

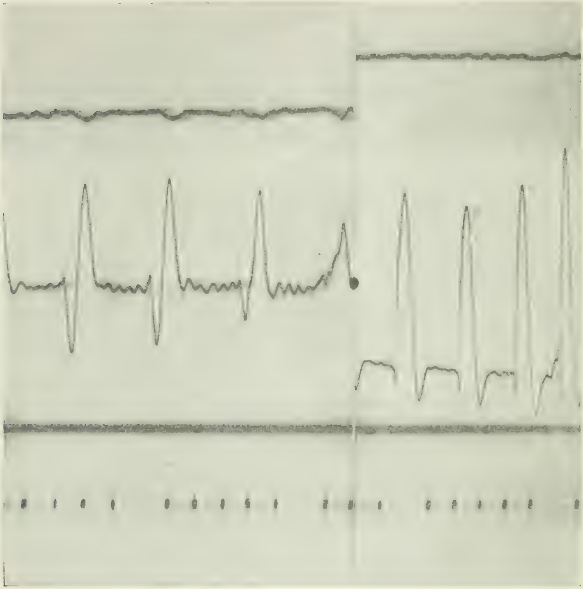

Fig. 6. To show shifting of the pace-maker. Electrograms on left from the lesser curvature near the cardia and preantrum on the greater curvature. The positive variation in the cardiac record and the predominately negative one in the preantral record indicate that the pace-maker is between them, sending waves in both directions. The records on the right taken a few minutes later from cardia and pyloric antrum show a different type of deflection. The animal was a dog. The tiny deflections on the cardiac record are respiratory—rate 36 per minute.

The negative (upward) variation seen in the figures shows that the waves are proceeding aborally over the stomach. Quite frequently,

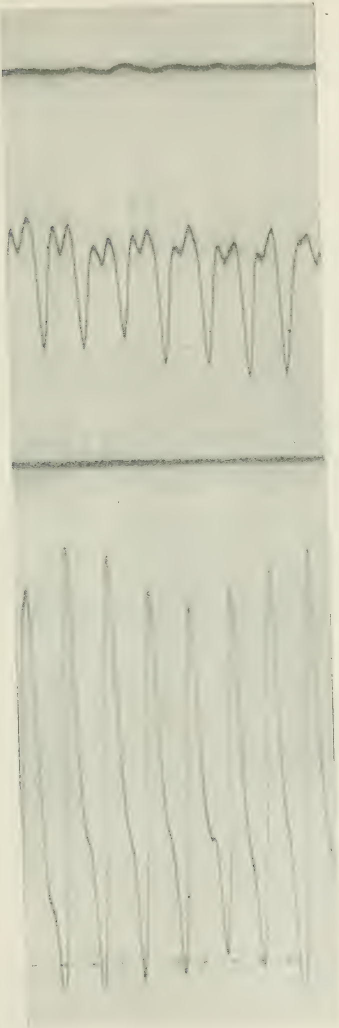

Fig. 7. To show again a positive variation in the cardia with a predominately negative variation in the preantral region. The uppermost record is a mechanogram from the fundus. The animal was a dog.

when the stomach is contracting slowly and irregularly; when the animal is sick, or when it is dying, it can be seen very clearly that the pace-maker shifts, sometines from beat to beat (see figs. 6 and 7). In one distempered dog the pace-maker shifted at times to the pylorus and reverse waves could then be seen running over the stomach.

One difficulty in analyzing the records arises in the fact that ordinarily several waves are going over the stomach at one time. It is much simpler in the heart where each cycle is distinct and separate. Fortunately, irregularities in the interval between waves often enable us to identify corresponding deflections at the cardia and at the pylorus.

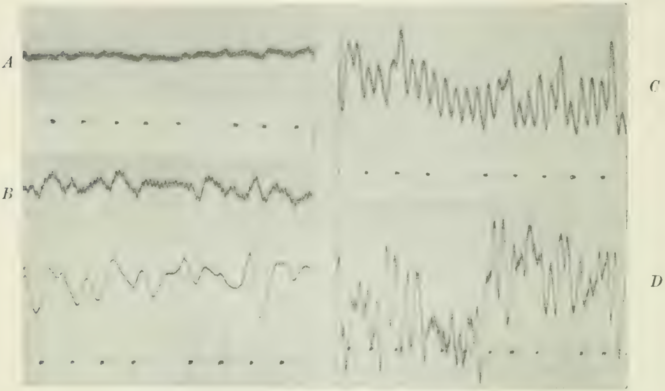

Fig. 8. To show: *A*, an electrogram from the gall bladder; *B*, electrograms from the liver and middle stomach; *C*, electrogram from the common duct; and *D*, electrogram from the first portion of the duodenum of a dog. The large deflections on the liver are generally derived from the stomach. In this case the small deflections are respiratory in origin, but they often come from the duodenum. The deflections in the common duct may also come from the duodenum but I am not yet sure of it, as the rates do not agree exactly.

Other observations. This technic may be very helpful in studying the motility of the gall-bladder and bile ducts although precautions will have to be taken to make sure that the deflections obtained are intrinsic and not derived from the adjacent stomach or bowel. Figure 8 shows a characteristic gall-bladder record from the dog. The contractions seem ordinarily to be derived from the adjacent duodenum but in some

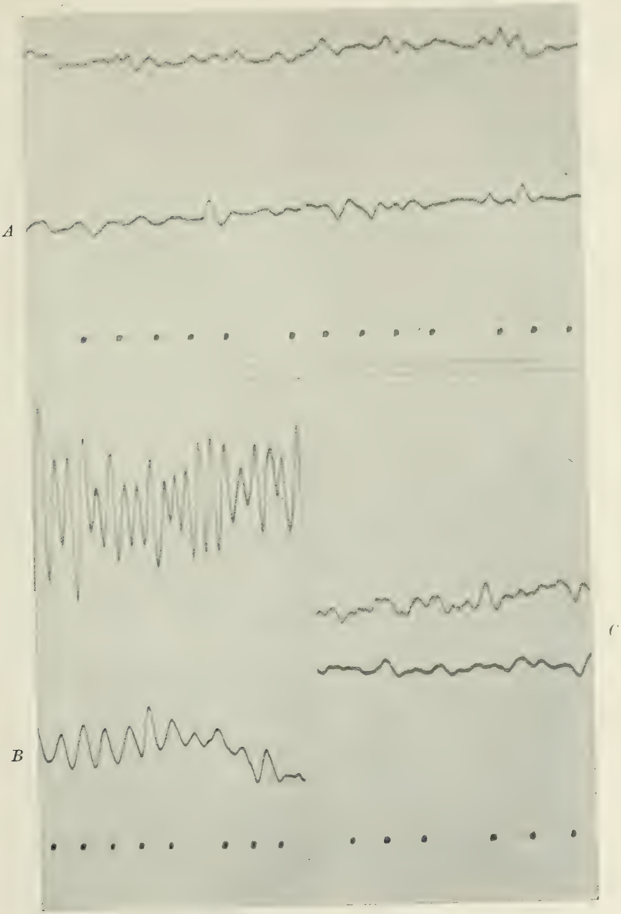

Fig. 9. Showing electrograms from *A*, the orad and middle regions of the rabbit's cecum; *B*, the terminal ileum and colon of a dog; and *C*, the colon and rectum of a rabbit. The time record represents 10-second intervals with a double gap every minute.

cases another, probably intrinsic, rhythm appears. The next curve was obtained from the liver and consists of deflections derived from diaphragm and stomach. The next curve is from the common duct. The rate of contraction is that usually found in the duodenum, but the accompanying record which was taken a moment later from the upper duodenum of the same animal has a very different appearance. The rabbit's cecum (fig. 9) shows small waves, derived perhaps from the ileum, with larger contractions superimposed once every minute or so.

It has always been supposed that there is little connection between the movements of the stomach and duodenum because the gastric waves stop at the pylorus. Many years ago I obtained considerable evidence which made me think that the gastric waves influence the activities of the duodenum and give rise to the rush waves which run from time to time down the length of the bowel. The new technic practically confirms these suspicions and shows marked electrical changes in the duodenum following the arrival of gastric waves in the pyloric antrum. The matter will be discussed more at length in a subsequent paper.

The demonstration of these electric currents, spreading from one part of the digestive tract to another and from the tract to adjacent glands like the liver, brings up some interesting problems. If Ralph Lillie and others are correct in their belief that the bio-electric currents have much to do with the institution and direction of chemical activities in the body it seems likely that these spreading abdominal currents might have something to do with that correlation of mechanical and chemical functions which is often attributed to hormones and nerves. At any rate, the subject would seem to be worth looking into a little farther.

We have done some work, stimulating the bowel between two pairs of electrodes, with the hope of demonstrating the "law of the intestine," but so far without success. These experiments will be continued and reported upon later.

SUMMARY

New electrodes and other apparatus have been devised, by means of which the action currents of the digestive tract can easily be studied. Two electro-enterograms and two mechanograms are recorded on the same drum.

Some evidence has been found of gradients of potential along the stomach from cardia to pylorus and along the bowel from duodenum

to colon. Orad regions are negative to cuadad ones. In the rabbit, the stomach and cecum, full of digesting food, are negative to other parts of the tract.

The elctro-enterograms obtained by attaching electrodes to various parts of the stomach and bowel resemble closely the mechanograms of the rhythmic contractions of bits of muscle excised from those parts.

The rates of rhythmic contraction correspond in the two types of records; and the rhythmic gradients in stomach and bowel have been demonstrated clearly again.

It is a striking fact that large currents are obtained from stomachs and bowels which, to the naked eye, show absolutely no peristaltic movement. Splanchnic stimuli which inhibit the contractions of the bowel have little or no effect on the rhythmic action currents.

In the stomach a negative variation travels from the cardiac region to the pylorus every 12 to 30 seconds. The cardiac region shows from 6 to 20 deflections a minute, superimposed sometimes upon other large tone-like changes which are synchronous with the gastric waves. The antral end of the stomach shows ordinarily only the deflections corresponding to the visible waves. Often these deflections are superimposed upon other extrinsic ones from the duodenum.

It is yet a question why only three waves a minute arise in a region which appears to be contracting from 6 to 20 times a minute.

At times it can be shown that the pace-maker shifts, even to the pyloric region.

Records are shown from the gall bladder, liver, common bile duct and cecum.

It has been found that there are marked electrical changes in the duodenum following the arrival of gastric waves at the pylorus.

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Reprinted from THE AMERICAN JOURNAL OF PHYSIOLOGY
Vol. 59, No. 1, February, 1922

EFFECTS OF THE GALVANIC CURRENT ON SEGMENTS OF INTESTINE

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Received for publication November 3, 1921

Alvarez and Starkweather (1) showed in 1918 that there is a gradient of metabolism in the muscle of the small intestine from the duodenum to the ileum. Per unit of weight and time the muscle from the duodenum gives off more CO_2 than the ileum. Similar gradients along the polar axes of lower forms of life have been shown to be associated with gradients in electrical potential (2); that is, the head end of the animal is negative (through the galvanometer) to the tail end. These differences in electrical potential cause the animal to resemble in a way a small magnet, the positive pole of which is at the head end and the negative pole at the tail. According to Child, this electrical polarity can account in part for the fact that such organisms, when placed in water through which a current is flowing, generally take up positions in the lines of force with their head ends toward the cathode. Some have felt that these positions are assumed because they give the animals least discomfort, but Loeb and others have shown that this so-called galvanotropism is due simply to the unequal or dissimilar effects of the entering and emerging currents on the muscles of the animal.

It occurred to us that inasmuch as the intestinal segments show a polarity, similar to that found by zoölogists in small water organisms, they might show a somewhat similar behavior when placed in the path of an electric current. We have presented elsewhere some evidence that there is an electrical gradient down the bowel from the duodenum to ileum, orad segments being negative to caudad ones (3). Alvarez and Starkweather (4) showed, moreover, as was to be expected, that conduction is better down the bowel with the gradient than orad, against that gradient. It seemed worth investigating, then, to see whether there would be any difference in the effects of a current traveling caudad down the gradient and one traveling orad, against it.

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[1] This work was made possible through the kindness of: Mr. T. E. Bibbins, Dr. W. B. Coffey, Mr. Mortimer Fleishhacker, Dr. John Gallwey, Mr. E. Clemens Horst, Dr. N. W. Jones, Mr. G. A. Mattern, Dr. Langley Porter, Dr. H. A. L. Ryfkogel and Dr. A. J. Zobel, who paid Miss Mahoney's salary during the past year.

In order to show differences in the effects of ascending and descending currents, two segments were put into a glass tube 3 cm. in diameter and 18 cm. long. The lower end was closed by a rubber cork pierced by a copper wire and a small tube for the air supply. Two segments of rabbit's small intestine, 3 cm. long, were suspended in this tube and fastened to heart levers so that they would write one above the other on a smoked drum. The tube was filled with Locke's solution aerated from below, and kept warm by immersion in a large water bath. Another copper wire dipped into the solution at the upper end of the tube. The current was supplied ordinarily by two dry cells in series, sufficient to produce definite effects on the bowel.

Sometimes the two segments were placed both with the cephalad ends up; sometimes one was placed with the cephalad end up and the other with it down. The direction of the current was then changed from time to time and the results were noted (see fig. 1). Although the segments seemed to writhe more actively, these movements were integrated in such a way as to appear on the drum as a lessening of amplitude with a slightly increased tone. No change in the rate of contractions could be detected; and up to this point the work lent no support to the gradient theory.

In the second set of experiments, segments of rabbit's intestine about 10 cm. long were put into a flat dish full of warm Locke's solution and the current from two dry cells was led in through copper wires. When the direction of the current was perpendicular to the long axis of the bowel the segment exhibited powerful writhing movements which showed a striking resemblance to those of an angle worm under similar circumstances. As was to be expected, the muscle on the cathodal side was stimulated so much more than that on the anodal that the loop was made to bend with its two cut ends approaching the cathode. In so doing it often was caused to turn over on account of the inextensible mesenterial attachment on one side. This would often bring another stretch of muscle onto the cathode side and more tumbling movements would result. When the segments were placed with the long axes in the path of current flow, the muscle on the end toward the cathode was stimulated most, with the result that in a typical experiment powerful waves began at that end and coursed to the other. The striking thing was that these waves could be sent alternately in the orad and caudad direction through the segment, simply by reversing the direction of the current.

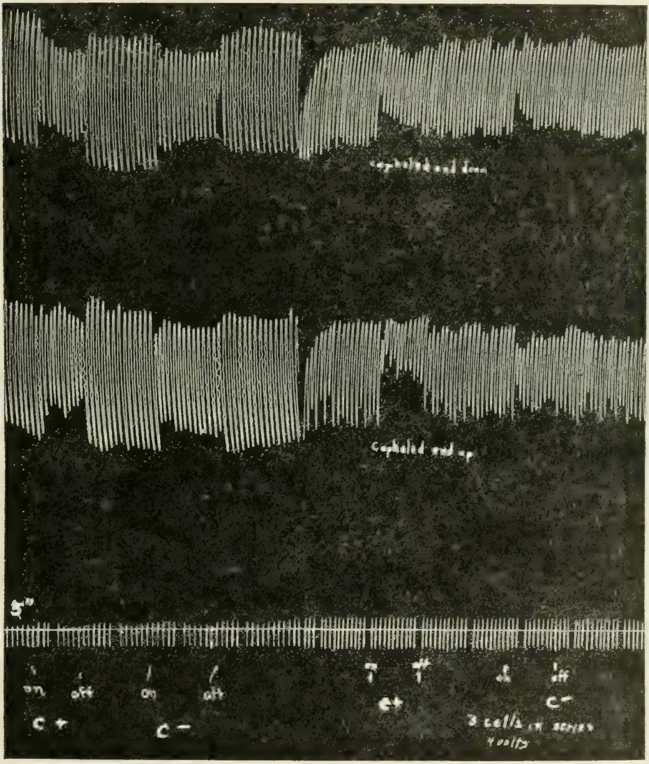

Fig. 1. Tracings from excised rabbit's jejunum (left) and ileum (right) beating in a long tube of oxygenated Locke's solution. On the left, the cephgalad ends were up; on the right, one segment had its cephalad end down and the other had it up. $C+$ means that the current entered at the upper end of the tube; $C-$ that it entered below.

It seems to us that these experiments lend support to the gradient idea of peristalsis because they show that the direction of the intestinal waves can be reversed by an electric current which reverses the gradient of muscular activity. Our work lends support also to the idea often expressed by Loeb, Parker, Garrey and others, that a worm or other lowly animal does not writhe about in certain ways under a stimulus because it is in pain and is hunting for a comfortable position, but because certain muscle groups are predominately affected by the stimulus. If we are to ascribe intelligence to the worm, we must look for it also in the excised bowel because that not only writhes in a wonderfully life-like manner when placed in the path of the electric current, but it shows a definite tropism.

SUMMARY

There is a metabolic gradient down the bowel from duodenum to ileum. Associated with this, there appears to be a gradient of electrical potential: in other words, segments of bowel are polarized as are many of the worms and plants.

When placed transversely across the path of a galvanic current short sections of rabbit's bowel showed a galvanotropism due to a greater stimulation of the longitudinal muscles on the cathodal side. When a segment was placed longitudinally in the path of the current peristaltic waves began at the cathodal end and ran to the anodal. The direction of peristalsis could be reversed by reversing the current.

When such segments were made to pull on a lever attached to one end, no difference was observed in the graphs obtained with ascending and descending currents.

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Reprinted from THE AMERICAN JOURNAL OF PHYSIOLOGY
Vol. 59, No. 1, February, 1922

THE MYOGENIC NATURE OF THE RHYTHMIC CONTRACTIONS OF THE INTESTINE

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IN COLLABORATION WITH

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Received for publication November 3, 1921

There are few statements in physiology upon which opinion is more unanimous than the one to the effect that the rhythmic contractions of the bowel are neurogenic, and due to impulses coming from Auerbach's plexus. We find that statement in almost all of the textbooks and articles which we have consulted in the last eight years. Such unanimity is rather surprising, but it is still more surprising when we learn that practically all the research work done on the subject points clearly in the opposite direction; and it is even more surprising when we learn that Magnus, whose early negative results are so generally quoted and accepted, later obtained positive results which quite upset his preliminary deductions. Unfortunately, it is easier for writers to quote from the English abstracts of Magnus' first two papers (1) than to wade through the seventy-one pages of German in the fifth Mitteilung (2) which records his success in getting denervated muscle to contract rhythmically.

In his first paper, Magnus describes the technic of using excised segments of intestine in Locke's solution and records a number of preliminary studies. In his second paper, he shows that the longitudinal muscle can be pulled off of the circular, carrying with it practically all of Auerbach's plexus. That is due to the fact that the nerve cells lie in grooves and deep recesses in the longitudinal layer. He studied his preparations after careful staining and found that only small fragments

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of the plexus remained adherent to the circular layer. These little pieces were so widely scattered and so badly damaged that he felt they could be disregarded. As the longitudinal muscle with the plexus attached contracted rhythmically and the circular did not, he was satisfied that the beat was neurogenic. Later, in his fourth paper (3), he showed that the active, plexus-containing strips and the quiet denervated ones reacted differently to electric stimuli. Some difficulties were met with in this study which it seems to us should have caused Magnus to doubt the sureness of his ground, but his troubles began in earnest when he started studying the effects of drugs on the segments. He was delighted at first to find that some of the drugs brought out distinct differences in the behavior of the plexus-free and plexus-containing segments, but later he discovered that certain substances such as barium chloride, strophanthin and pilocarpin would bring out rhythmic contractions even in the denervated segments. This led him to the conclusion that, although the preparations of circular muscle which he had been using were denervated sufficiently for most types of physiologic study, they were not denervated sufficiently for pharmacologic work. In order to remove all traces of the plexus, he seared the outer surface of the circular muscle with crystals of nitrate of silver; and when strips treated in this way failed to contract rhythmically even in the presence of the above-mentioned drugs, he felt that he had gotten rid of all traces of the plexus. Unfortunately for his peace of mind, he found next that physostigmin would bring out beautifully rhythmic contractions even in the partly charred strips which he was sure were plexus-free. These contractions are shown in figure 1, *A*, copied from his paper. As he could go no farther in the matter of denervation, and as physostigmin brought out rhythmic contractions in strips which reacted in a typically "denervated" way to other tests, Magnus had to admit that the contractions were myogenic. He found, moreover, that physostigmin acts in the same way on the denervated muscles of sipunculus nudus (4). In that animal there can be no doubt about the completeness of the denervation because the ventral nerve cord is easily visible and can be dissected off in its entirety.

In 1914 Gunn and Underhill (5) repeated this work of Magnus, using a better technic. They felt that they could cut down the severity of the shock of dissection by tearing the strips apart under iced Locke's solution, which acted as an anesthetic. As they had good reasons for believing that the silver nitrate used in searing the outer surface of the circular layer would diffuse into the tissue and damage the muscle throughout the strip, they avoided using it by taking for their experiments only a few fibers from the mucous membrane side. In this way they obtained muscle which certainly would seem to have been plexus-free, and which contracted rhythmically without the help of any drugs (see fig. 1, *B*, copied from their article). They were impressed also with the fact which has always interested us and that is that the segments continue to contract rhythmically for 4 or 5 days after removal from the animal. We have records showing that canine and human intestinal segments will contract even more regularly on the third day than on the first. The rate of contraction in the rabbit's small intestine is sometimes even faster and more regular on the second day than on the first (6). As it has been shown experimentally that some sympathetic ganglia fail to conduct stimuli within a few minutes after the stoppage of the circulation, it does not seem reasonable to assume that the function of the intestinal ganglia can *improve* after 48 hours of anemia. A similar persistence over several days of the rhythmic contractions in the retractor penis of the dog and in the cat's bladder convinced De Zilwa (7) and Stewart (8) that the underlying mechanism must reside in the muscle.

Another interesting observation of Gunn and Underhill was that the ability of the muscle to contract rhythmically; its ability to respond to drugs like atropin, pilocarpin and adrenalin, which are supposed to act on nerve endings; and its ability to respond to barium chloride which is supposed to act on muscle, were all lost together on the fifth day when the tissue apparently died. We have repeated these experiments and have also failed to obtain any evidence of nerve endings dying on one day and muscle on another.

Under the circumstances, Gunn and Underhill felt that there could be no doubt about the myogenic origin of the rhythmic contractions. Bayliss and Starling (9) had come to a similar conclusion many years before. They found that while cocain, applied to the surface of the gut, abolished the myenteric reflex, it actually improved the amplitude and regularity of the rhythmic contractions. As nicotin had the same effect when injected repeatedly and in large doses, they had good reasons for believing that they had paralyzed the plexus; that the myenteric reflex is due to the nerves; and that the rhythmic contractions are due to the muscle. Although it is best not to put too much trust in physiologic deductions based upon the assumed selective actions of poisons, it does seem to us that this evidence must be faced. Certainly we cannot go on as we have been doing, quoting this work to show that the progress of food through the bowel is based on the nervous mechanism, and Magnus' early work to show that the rhythmic contractions are also based upon it. A brief analysis of the problem will show that if Bayliss and Starling were right Magnus must have been wrong, and vice versa.

Recently Hammett (10) has shown that excised segments of rats' bowels will continue to contract rhythmically in a 1:4000 solution of cocain; and we have found that similar segments of rabbits' ileum will continue beating in 1:360 novocain solution. Fulton (11) has shown beautifully that novocain acts on the "intermediate substance" between nerve and muscle, and that this substance is apparently to be found in the nuclei of the muscle cells. He shows that novocain has little effect on the heart beats of the embryo chick, either before or after nerves grow into it. What it does is to block the nerve endings, so that stimulation of the vagus is no longer effective. This pharmacologic evidence all points again to the myogenic origin of the intestinal contractions.

When one remembers how common rhythmical processes are in nature, it is hard to understand why physiologists tend so often to ascribe those observed in muscle to that highly specialized organ, the nervous system. Thus Ostwald (12) showed that curves closely resembling those traced by a frog's heart can be produced simply by attaching an ordinary tambour to a flask in which hydrochloric acid is acting on chromium, with the evolution of hydrogen. Rhythmic changes take place in the metal which make it alternately resistant and subject to attack by the acid (see fig. 1, *c*, copied from Ostwald's article). Similar rhythmic changes occur in the rate of catalytic decomposition of hydrogen peroxide in contact with mercury, owing to the alternate formation and dissolution of a surface film of "peroxidate." In both of these reactions electricity plays a large part, much as it does in the animal body. Lillie (13) has been able to produce rhythmic "action currents" by putting two piano wires in nitric acid and connecting them to a galvanometer, and we have observed similar currents arising in calomel-electrodes that have been used too long.

When water drips at regular intervals from a reservoir we have a rhythmic response to a constant force, and Forbes and Gregg (14) have remarked upon several instances of this type of reaction in the body. Quincke (15) produced rhythmic alterations in the shape of an air bubble, trapped under water, and placed in the path of a fine stream of alcohol. The pulsation of the bubble closely resembles that of a heart, and is due to alterations in the surface tension. Passing onward from

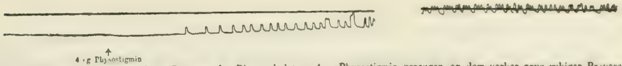

Fig. 22. (Versuch 153 a.) Plexusfreies Praparat der Ringmuskulatur. 4 cg Physostigmin erzeugen an dem vorher ganz ruhigen Praparat rhythmische Spontanbewegungen mit allmahlicher Tonussteigerung.

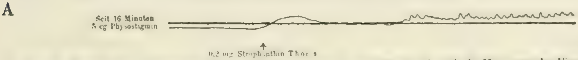

Fig. 23. (Versuch 156 A.) Plexusfreies Praparat der Ringmuskulatur. Das Prapart steht seit 16 Min. unter der Einwirkung von 5 cg Physostigmin, ohne Bewegungen zu zeigen. Auf 0,2 mg Strophanthin Thoms zunachst glatte, charakteristische Strophantinkontraktion, darauf Einsetzen rhythmischer (Physostigmin-)Bewegungen.

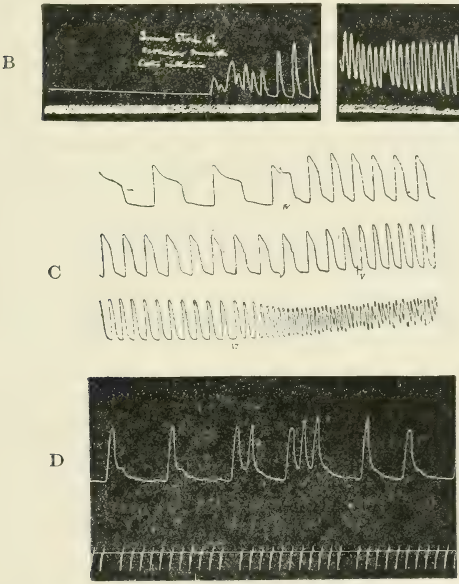

Fig. 1. *A*, Copy of Magnus' illustration showing rhythmic contractions in plexus-free preparations. *B*, Copy of Gunn and Underhill's illustration showing the same thing. *C*, Copy of Ostwald's illustration showing rhythmic changes in a relatively simple inorganic chemical reaction. Note the increase in rate with a rise in temperature. *D*, Rhythmic contractions obtained from strips of muscle removed from the inner face of the circular layer of the dog's intestine, the longitudinal layer, with the plexus, having previously been removed. The time record represents 5-second intervals.

these relatively simple chemical and physical phenomena, we find that Bose (16) has obtained beautifully rhythmic tracings of the contractions of the leaflets of certain plants. A little higher in the scale, we find animal tissues, not yet muscular in structure, but possessing the power of rhythmic contraction. We need only mention cilia and the tails of spermatozoa.

The heart beat seems to originate in muscle or muscle-like tissue except in the Limulus, a very primitive type of crab; and even in that animal Garrey (17) has shown that the denervated muscle will contract rhythmically if put into M/2 NaCl. Gaskell (18) found that the heart of a leech can be removed entirely from the nervous influences without stopping its activity. The heart of a tunicate will continue to beat when separated from its ganglion and even when removed from the body (19). Luchsinger (20) found that the veins in a bat's ear will contract rhythmically if they are perfused even as late as 50 hours after death. The heart of an embryo chick beats after 29 hours of incubation, although nerve cells do not reach it until the sixth day (21); and even the isolated heart muscle cells in tissue cultures have been observed to beat (22). The same thing was seen by Lewis and Lewis (23) in cultures of smooth muscle and by Lewis (24) in cultures of skeletal muscle. The retractor penis of the dog is made up of smooth muscle; it has no ganglia (25) and yet it contracts rhythmically when excised (26). Loeb (27) has shown, moreover, that even striated muscle will contract rhythmically in the proper concentration of NaCl. Nice and Neill (28) have recently called attention to rhythmic changes in diaphragm muscle. Hydromedusa stops pulsating when the nerve net is excised and this was supposed to prove the nervous origin of the beat until Loeb (29) showed that the muscle will start up again if the concentration of the seawater is changed. Romanes (30) found, moreover, that the nerveless centers of other types of jellyfish will sometimes beat in seawater after the shock of the operation has worn off.

This review of the literature shows that rhythmic contraction is a property inherent in muscle and muscle-like tissues; and there is good reason to believe that it is brought about rather simply as the result of recurring cycles of chemical activity. Similar metabolic cycles might be present just as well in nervous tissue; and there is no doubt that in some situations, as in the bell of the medusa or the heart of Limulus, the normal pacemaker is to be found in ganglion cells. The only article that we can find in support of the idea of a neurogenic origin for the intestinal contractions is that by Yanase (31) who observed peristalsis in embryo guinea pigs only after the 26th day, when the myenteric plexus and the longitudinal muscle appear. This, however, is negative evidence which may be objected to for a number of reasons.

Experimental work. Remembering that the dog's intestine often contracts better on the second or third day after excision, we thought it well to test the rhythmicity of circular muscle, stripped away from the longitudinal, 24 hours after the bowel had been placed in the icebox. Following Gunn and Underhill, we used only the inner half of the circular layer lying next to the mucous membrane which was also stripped off. We were able to get rhythmic contractions from such muscle which we felt sure had been freed from all traces of the nerve net. In some cases we were able to show with the microscope that considerable tissue had been left between the plexus and the muscle fibers removed for the experiment. It was not difficult to get rhythmic contractions from such segments after adding a trace of barium to the Locke's solution. Without the barium we had to wait sometimes for 6 or 7 hours before contractions appeared.

Our recent work with delicate galvanometers has shown that strong rhythmic action currents are being developed in segments of intestine which, so far as the eye can see, are absolutely quiet (32). This observation brings up some interesting questions. It suggests that rhythmic chemical processes are continually going on in the muscle; processes which, from time to time, give rise to the physical contractions. The presence of a little barium or physostigmin seems to act like the clutch in an automobile which causes the energy of the engine to appear at the back wheels as motion. Similarly in the heart, Mines (33) found that the withdrawal of calcium stopped the beats but not the action currents. De Meyer (34) thinks that in the heart there is no relation between the beat and the intensity of the action current because the current remained unchanged when the heart was stopped by clamping the aorta. We have found that splanchnic stimulation, or the painting on of adrenalin, will stop the intestinal contractions and still leave the currents. It is clear, then, that we cannot always say, when a muscle is not beating, that it has lost its source of rhythmic stimuli. In the language of the simile, the engine may still be running but the clutch may be out. Some may still suspect that the rhythmic engine resides in the nerve net but that seems unlikely on account of the intensity of the currents produced. Our impression is that current intensities are more or less proportional to the volume of muscle involved, i.e., the currents in a dog are much more powerful than those in a rabbit. Yet there is remarkably little difference sometimes between the action currents of an immobile and an actively segmenting bowel. A glance at figure 2 will show that the intensity of the action currents in an excised segment of rabbit's ileum, rendered absolutely immobile by adrenalin, is about four-tenths of the intensity of the currents in the active bowel. In some experiments it was nearer six-tenths. If the currents of the inactive bowel were generated in the tiny nerve net and those of the active bowel in the net and muscle together, we would expect a much larger difference. In passing, it is of interest to note that this adrenalin which stopped the rhythmic movements of the muscle increased the rate of the rhythmic action currents from 10.5 to 14 per minute.

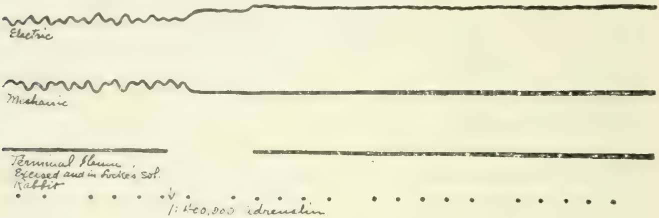

Fig. 2. Upper record shows the electric changes, the lower shows the mechanical contractions. The break in the signal line shows when adrenalin was added. The time record represents 10-second intervals. A segment of rabbit's ileum was suspended in a beaker containing 400 cc. of warm aerated Locke's solution. The currents were led off from specially designed calomel electrodes. The contractions of the gut were transmitted by tambours to the photographic device described elsewhere.

In closing, it should be pointed out that the question of the neurogenic or myogenic origin of the contractions is a more or less academic one because under normal conditions the muscle and nerve fibers are intimately associated and are designed to work together. There is no question but that in most cases the muscle works better for the presence of its nervous plexus. The immediate effect of the removal of the nerves is shock and spastic paralysis; and, when that is recovered from, we cannot expect the tissue to work quite so well as it did normally. The conduction from muscle bundle to muscle bundle must remain impaired.

Our reasons for reviewing the subject so at length are twofold: first, to point out certain errors which have crept into the literature; and second, to call attention to the importance of studying the muscle as well as the nervous tissue. We feel that the tendency to push the explanation for vital phenomena out of the organs in which they might be studied and into elusive little ganglion cells is often unfortunate, and conducive more to stagnation of thought than to advancement of knowledge.

SUMMARY

Most writers quote Magnus' early work to the effect that the rhythmic contractions of the intestine are neurogenic in origin. Few seem to know that in later experiments he was able to get good contractions from well denervated muscle.

The positive results were subsequently confirmed by Gunn and Underhill, and now by the writers. They are in agreement also with the findings of Bayliss and Starling.

It is shown now, moreover, that even if a bit of intestinal muscle fails to beat, it does not follow necessarily that it has lost its source of rhythmic stimuli, because wave-like action currents can, in many cases, still be demonstrated. These currents are so nearly equal in intensity to those observed in active segments that it can hardly be argued that they arise in the nervous tissue, the mass of which is small.

A review of the literature on rhythmic processes in nature shows that there is no need for looking beyond the muscle for the source of the rhythm.

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NEW METHODS OF STUDYING GASTRIC PERISTALSIS *

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SAN FRANCISCO

It is hard for a moment to realize that our knowledge as regards gastric physiology is today in much the same stage as it was as regards cardiac physiology back in the early seventies, before the sequence of the beat in sinus, auricles and ventricles had been studied by means of graphic records. In those days the research workers simply watched the slowly beating hearts of dying amphibians and tried to understand the movements which they saw, much as we, today, try to understand the sequence of the gastric waves as they appear before us on the fluorescent screen. To be sure, we obtain graphic records by taking plates, but such plates are snap shots which cannot help the physiologist very much. A few roentgenologists, notably Rieder in Europe and Cole in America, have secured interesting serial roentgenograms of the stomach; but the technic has been so difficult and the apparatus so expensive that, so far as I know, the method has not been used in any physiologic laboratory. Cannon did much of his epoch making work with the roentgen ray; but his records consisted mainly of tracings of the shadows which he saw on the screen. Carlson and his students have done an enormous amount of work with balloons inserted into the stomachs of men and animals; but, as they admit, such balloons slip about; it is hard to say just where they are; and they certainly cannot give us information as to the way in which the waves travel over the organ. Recently, Wheelon and Thomas [1] have invented a type of anchored balloon with the help of which they have obtained interesting records from the pyloric region.

It has always seemed to me that an exact knowledge of the movements of the stomach could be secured best

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* From the George Williams Hooper Foundation for Medical Research and the Department of Medicine, University of California Medical School.

* Read before the Section on Gastro-Enterology and Proctology at the Seventy-Third Annual Session of the American Medical Association, St. Louis, May, 1922.

[1] Wheelon, H., and Thomas, J. E.: J. Lab. & Clin. Med. **6**: 124 (Dec.) 1920; Am. J. Physiol. **59**: 72 (Feb.) 1922.

by fastening light recorders very gently to the outer surface of the organ; and in 1914, with the help of a simple little enterograph, I was able to obtain simultaneous records from the middle region and the pyloric antrum of a few rabbits and cats. Several years before, Hofmeister and Schütz,[2] and Sick and Tedesko[3] obtained somewhat similar records by fastening light heart levers to two or three places on the excised stomachs of laboratory animals. Such stomachs, kept in warm, oxygenated Locke's solution, will often show considerable activity for hours; and I have secured a number of interesting kymographic and electrographic records from them. The technic is naturally much easier than that required when we try to record the small, weak contractions of the cardiac end of the intact stomach, situated as it is high up under the diaphragm and moving constantly with respiration. The results obtained with the organ in situ are so much more trustworthy, however, that we must continue to grapple with the technical difficulties which stand in our way.

A number of these difficulties have now been conquered sufficiently so that we can get simultaneous mechanical records from five or six parts of the stomach. I shall not attempt here to describe the apparatus, because it is still in the stage of constant change and improvement. Another way of attacking the problem is to detect and make records of the delicate action currents which can tell us so much about what is going on in the muscle. Should a study of these currents in the stomach yield but a tenth of the information which a similar study has yielded in the fields of heart physiology and pathology, the science of gastro-enterology will be put on a new footing. Much of the technic for getting electrogastrograms and enterograms has already been worked out, and two brief reports on the subject have been published.[4] One of them contains a reproduction of the first human electrogastrogram to be taken.

Naturally, in the first stages of such an investigation, the interpretation of the records is difficult, and it is

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quite essential that the electrical changes be compared with mechanograms made at the same time and at the same place. Although this need has added greatly to the technical difficulties which already were troublesome enough, we have during the last few months secured a number of records like those in Figure 1, which show simultaneous mechanograms and electrograms from two parts of the stomach or bowel. They show that in most cases the electrical wave looks very much like the corresponding mechanical one. The two run closely together; and after a while we shall probably be able to read the meaning of the electrical records so clearly that we can dispense with the mechanical ones and can continue with the less troublesome electrical technic.

WHAT CAN WE LEARN WITH THE NEW TECHNIC?

The next question is: What is being learned with these methods? In the first place, we are learning something about the place of origin of the gastric waves and how they travel over the stomach. As I pointed out in the previous articles, many of the records suggest strongly that the waves ordinarily arise at a point on the lesser curvature near the cardia. That may perhaps be the normal pacemaker, which sends out ripples which deepen into waves in the lower third of the stomach. Other records show signs of a shifting of the pacemaker, while others, again, show that two parts of the stomach are contracting with independent rhythms. Just as in the heart, so in the stomach, there appear to be gradations from transient blocks to complete dissociations. Such blocks have been found most frequently at the junction between the body of the stomach and the pyloric antrum; but a number of the records show that they can occur elsewhere. In the excised stomachs of rabbits, cats and dogs we can often see the lower end of the esophagus contracting powerfully and rhythmically, but only occasionally sending a wave over the adjacent stomach. Other records show what I have frequently observed on the fluorescent screen, and that is, a series of contractions which do not spread over the stomach, but spring up and apparently die away in the same place.

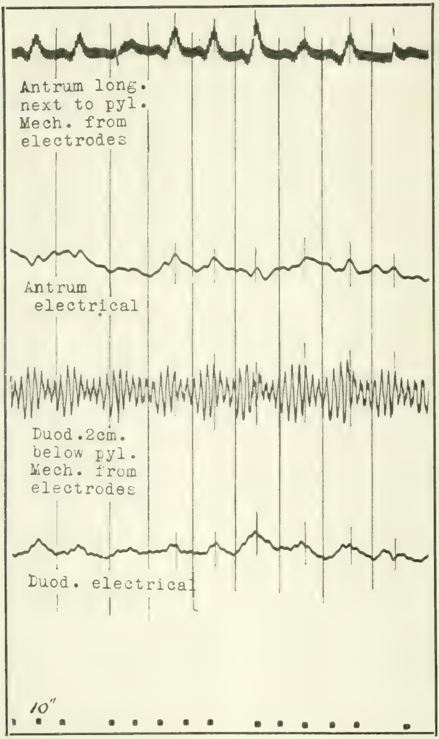

Fig. 1.—Electrograms from the antrum next to the pylorus and from the duodenum 2 cm. below, in a rabbit. The mechanograms, recorded simultaneously with the electrograms, represent the movements of the electrodes as the muscle contracted. An upward movement on the mechanical records signifies contraction, and in the electrical records it signifies a negativity of the orad electrode. The time record indicates intervals of ten seconds. The small waves on the first antral record are respiratory in origin. Note the increase in amplitude of the duodenal contractions with the arrival of each gastric wave at the pylorus. The vertical lines have been drawn to indicate synchronous points on the record.

Another interesting observation is that a contraction near the cardia or in the preantrum may be followed almost instantly by a contraction of the pyloric antrum or of the stomach as a whole. This "systole" was seen years ago by Cole on his serial roentgenograms of human stomachs; but as the pacemaker seems often to give rise to a systole and peristaltic wave at the same time, the double nature of the contraction can hardly be recognized on the screen; and no one has felt inclined to pay much attention to Cole's observation. I admit that when Cole showed me his plates I thought that the phenomenon must be rare and of little

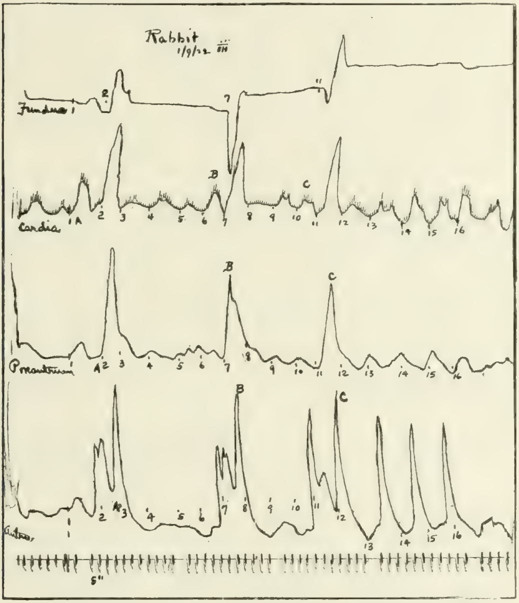

Fig. 2.—Mechanograms from the fundus, cardia, preantrum and antrum of a rabbit's stomach. The small waves just visible on the second record are respiratory in origin. The interval on the time record is five seconds. The figures represent corresponding points on the four records, while the letters *A*, *B* and *C* show that certain contractions were part of one peristaltic wave.

significance. Now that I find it so commonly in my records, I am glad to express my apologies to Dr. Cole and my willingness to believe that many things appear on the fluorescent screen which we are not quick enough or prepared enough to see. For that matter, watching three waves going over the stomach at one time is like watching an expert at the shell game: it is not likely to yield exact or trustworthy information.

But to return to the systoles: It may be that they interfere sometimes with the emptying of the stomach because, as can be seen from Figure 2, the waves marked *A, B* and *C,* which were seen to travel over the stomach, reached the pylorus while the muscle there was partly contracted. The record of the antral contraction often shows two components, and sometimes even three. The first one may represent the tendency of the muscle to contract with its own natural rhythm; the second may represent a systole, while the third represents the arrival of a peristaltic wave. These multiple activities sometimes make it almost impossible to interpret the electrograms. If we glance again at Figure 2, we see that in each set of contractions there is a second cardiac one which fails to give rise to any appreciable peristaltic wave. This second cardiac hump is so often synchronous with the arrival of the peristaltic wave at the pyloric antrum that I have often obtained the impression that a systolic type of impulse has shot backward. Hence, for convenience' sake, I have come to call this well defined and easily recognizable type of gastric activity "shuttle rhythm." Sometimes the impulse seems to shoot back so quickly that the two cardiac humps fuse to a greater or less degree.

In other records, we find a series of single gastric systoles with either the cardia or some other part of the stomach slightly in the lead. In some excised stomachs, we saw what appeared to be peristaltic waves; but the records showed that they were more like systoles. All parts of the stomach began to contract at the same time; but as it took the muscle in the lower end of the stomach a progressively longer time to get under way, the appearance was that of a wave spreading downward.

Figure 3 shows a common type of peristalsis in which some antral contractions are much more power-

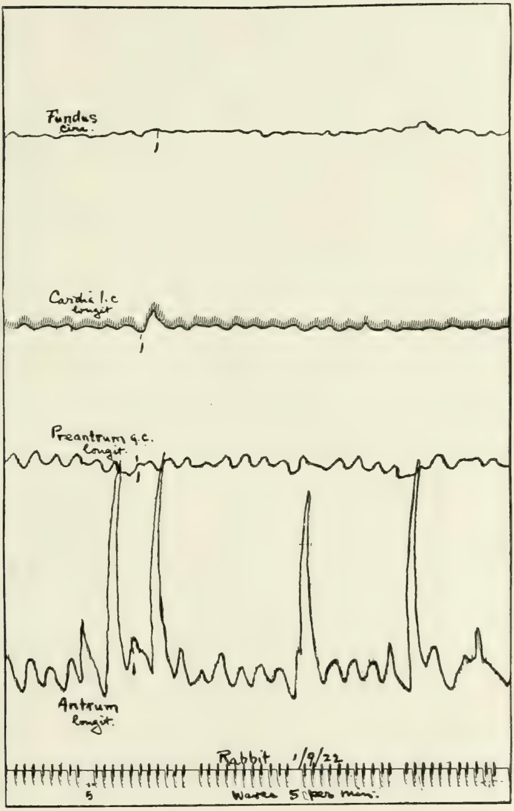

Fig. 3.—Mechanograms from the fundus, cardia, preantrum and antrum of a rabbit's stomach. The time interval represents five seconds. Note the large antral contractions which seem to arise locally.

ful than others. We see that commonly in man; and the frequency with which these large waves appear undoubtedly has much to do with the rate of emptying of the stomach, and perhaps a good deal to do with the production of pain and distress in diseased states. The graphs bring out the interesting fact that this increase in activity must often be purely a function of the pyloric antrum, because one can see no sign of corresponding differences in the size of the waves in the body of the stomach a centimeter above. There are some records which suggest strongly that the variation in the size of the antral contractions may be an interference phenomenon between the fundus rhythm and the inherent rhythm of the antral muscle. Previous experiments have shown me that the excised antral muscle will contract about three times a minute. If this should happen to be the rate of the waves coming down over the stomach, the antral contractions could easily be uniform in size and strength. When, however, there is a slight difference in rate, it might account for the presence of larger waves when the rhythms coincide and smaller ones when they conflict. Irregularities in the recurrence of these beats must be expected because other rhythmic components will come in by way of the duodenum.

This difference in the activities of the two functional parts of the stomach, the "crop" and the "gizzard," is brought out even more sharply in the records showing blocks and dissociations. A kymographic record of these blocks was shown in one of the preceding articles, and two electrograms depicting the same thing are shown now in Figure 4. Simultaneous mechanograms and electrograms made so far show that when the currents are absent from the antrum the contractions are also absent; but in one case when, for a short time, no contractions could be detected, weak action-currents still appeared at regular intervals. So far I have not seen anything on the records which looks like the common cardiac 2:1 or 3:1 block. The gastric blocks look more like those which we see in Stokes-Adams disease.

Another way in which the differences between the physiologic characteristics of the fundus and pyloric antrum can be brought out is by stimulating the vagus nerve. In a few such experiments done in my laboratory on rabbits, it was a striking thing to see the recording lever attached to the body of the stomach shoot upward (indicating contraction) while the lever on the antrum dropped. Other experiments have suggested that the pyloric antrum is more sensitive to all inhibitory influences than is the body of the stomach.

To me, one of the greatest surprises has been the discovery that any group of muscle fibers in the stomach wall may respond at the same time to two or more rhythmic activities. Thus, it may show small contractions perhaps every five seconds. These probably represent the local inherent rate, i. e., the rate at which the muscle contracts when excised and put into warm oxygenated Locke's solution. Besides the small contractions there may be slow tonus waves coming every minute or two. These tonus changes seem to be going on constantly throughout the digestive tract, and I believe that they must play a large part in regulating and directing peristalsis. The few records which we have obtained of these rhythms suggest that they can sometimes be captured and made to follow

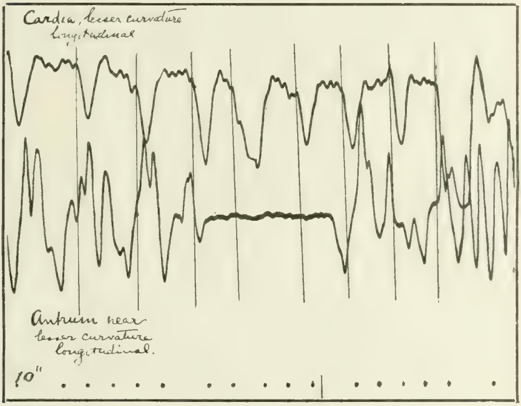

Fig. 4.—Electrograms from the cardia and antrum of a cat. The small deflections on both records are probably respiratory in origin. Note the blockage of two waves. The main deflection in the cardia's record is a positive one, suggesting that the pacemaker lay between the two electrodes placed on the lesser curvature next to the cardia.

other regularly recurring impulses whose rate is not too widely different. This can be seen at the pyloric line where the duodenal tone rises shortly or immediately after the arrival of each gastric wave. The interesting point is that during intervals of complete gastric quiet, when even the action currents are absent, the duodenal tonus rhythm may continue unchanged, showing that it can be just as independent as the similar rhythms which can easily be demonstrated in the ileum and colon. Furthermore, when the stomach starts up again it does not always succeed immediately in capturing and dominating the duodenal tonus rhythm. As will be seen from Figure 1, this tonus wave shows itself sometimes only as a rhythmic increase in amplitude of contraction. It appears also in the electrograms as an increase in the strength of the action current.

But to return to the question of multiple rhythms in the stomach: First, there are the small, frequent waves analogous probably to the rhythmic contractions of the bowel; secondly, there are tone changes like those in the bowel; and thirdly, there are peristaltic waves corresponding probably to the peristaltic rushes in the small bowel and to the mass movements in the colon. These compound movements are seen most often in the body of the stomach, but we have seen signs of them sometimes in the antral records. The electrograms from the fundus generally show a series of small respiratory deflections, while those from the antrum often show a small deflection synchronous with the duodenal contractions. These are probably transmitted by conduction through the tissue, although I have seen similar little humps on some of the mechanical records from the antrum. These, however, probably represented little pulls on the gastric recording apparatus by the adjacent bowel.

One more observation requires comment at this time, and that is that many of the mechanical records from the fundus show relaxations corresponding to the passage of the peristaltic waves. The fundus has a little different embryologic development from the rest of the stomach, and there are a number of indications that the working out of its rhythmic activities will be quite a problem in itself. In many of the records (both mechanical and electrical) from the lower end of the stomach there are signs of a preliminary relaxation before the contraction; but it may be that this is due simply to a passive stretching of the muscle as it is pulled on by the contracted area just above. Certainly a large proportion of the records show no sign of a preliminary relaxation such as we would expect if the "law of the intestine" were an important factor in regulating peristalsis.

Enough has been said, it seems to me, to show that large gaps exist in our knowledge of gastric peristalsis. It is this lack of exact information which has been such a handicap to the surgeons in their efforts to cure gastric and duodenal ulcers. They have had to learn by sad experience not to make such things as long loop gastro-enterostomies and V-shaped excisions on the lesser curvature, but just why they still do not know. I sometimes think of the troubles the surgeons would have fallen into if they had been able to cut out infarcted areas in the heart and to short-circuit stenosed valves as easily as they operate on the stomach; how many disturbances of conduction they would have produced and how many patients they would have sacrificed until they had learned empirically to keep away from the bundle of His and its important branches. This only emphasizes the fact that the physician is too often called on to attempt repairs on a structure the normal workings of which he has not yet been able to understand. Logically, we should learn all about the physiology of an organ before we attempt to repair a diseased one; but unfortunately the demands of the sick are too urgent; we must do our best with what little knowledge we have; but at all times we must keep our eyes fixed eagerly on the laboratories from which must come some day the needed help and guidance.

SUMMARY

Our knowledge of gastric physiology is still largely in the crude stage of purely visual observation.

The problem is now being attacked with methods for obtaining multiple and simultaneous mechanical and electrical records from the wall of the stomach.

Something has been learned about the gastric pacemaker; about different types of rhythmic activities;

about gastric "systoles"; about "blocks" and dissociations of activity between the body of the stomach and the pyloric antrum.

Stimulation of the vagus produces quite different effects in the fundus and in the antrum.

It is surprising to find areas on the stomach in which the muscle is responding at one time to two or more sets of rhythmic impulses.

Some observations have been made on the influence of the stomach on the duodenum.

The records generally show little sign of the "law of the intestine."

The fundus sometimes appears to relax while the rest of the stomach is contracting.

The large gaps which exist in our knowledge must be filled if the surgeon is to operate safely on diseased stomachs.

177 Post Street.

Reprinted from The Journal of the American Medical Association
Oct. 14, 1922, Vol. 79, pp. 1281-1284

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American Medical Association, 535 N. Dearborn St., Chicago

Reprint from the California State Journal of Medicine,
August, 1922

IS THE MELTZER-LYON TECHNIC FOR THE DIAGNOSIS AND TREATMENT OF GALL BLADDER DISEASE AN ASSET OR A LIABILITY?*

By WALTER C. ALVAREZ

PART I.

In 1917 Meltzer suggested, first, that inasmuch as the sphincter at the end of the common duct presumably must relax when the gall bladder contracts, if the contents of the gall bladder are to be extruded into the duodenum, the activities of the sphincter and the bladder must be coordinated and under the control of the "Law of Contrary Innervation." Second, if this be true, magnesium sulphate, when it relaxes the sphincter, must theoretically cause a reflex contraction of the gall bladder. Third, if magnesium sulphate were to be injected into the duodenum, it might help in overcoming jaundice and biliary colic.

The third suggestion was immediately put to the test by Vincent Lyon of Philadelphia. He observed that when the duodenal tube is first passed into the bowel, little or no bile can be withdrawn. Shortly after the injection of the magnesium sulphate, yellow bile appears; and shortly after that, there appears a darker and sometimes thicker bile. He assumed that the darker bile must come from the gall bladder, and the next step was to diagnose cholecystitis by the finding in this bile of pus cells, crystalline material and bacteria. Another step was to try to cure cholecystitis by repeated "drainages."

Certainly if Lyon is right in his contention this new method of diagnosis and treatment will be a great asset to the medical profession. If, however, the method is not based on a solid foundation; if it will lead to the making of wrong diagnoses and the performance of unnecessaray operations; if it will cause physicians to waste their time and their patients' time and money; and if it will encourage patients to go on with futile medical treatment until old age, starvation, myocarditis and other complications bar the way to surgical relief, it will be a liability. As I have seen of late a number of cases in which the use of the method was ill-advised and its results unfortunate, it seems to me that we must not drift along using "biliary drainage" simply because it is being done, but as scientific physicians we must stop for a few minutes to see how much grounding it has in physiologic fact, and how much likelihood there is of its ever being able to clear up the diseased conditions which we find in cholecystitis.

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* Read before the Section on General Medicine at the Fifty-first Annual Meeting of the Medical Society of the State of California, Yosemite Valley, May, 1922.

A review of the large literature which has already grown up about the subject shows that, for the most part, the clinical reports are very favorable. That is to be expected with any new form of treatment. On the other hand, nearly all the reports on work done in an attempt to determine the accuracy of Meltzer and Lyon's physiological contentions have served to cast doubt upon them and upon the method derived from them. In the first place, Meltzer's original theorem of a contrary action has been pretty thoroughly discredited by the finding that a number of substances besides magnesium sulphate will produce the typical flow when they are injected into the duodenum. As most of these drugs do not relax the sphincter, it cannot be argued that they will reflexly stimulate the gall bladder. Furthermore, it cannot be shown on either animals or men that the flow of bile produced by magnesium sulphate is accompanied by a contraction of the gall bladder. It seems clear now that that organ is a weak and sluggish one which never contracts down like the urinary bladder does, even when it is stimulated directly by strong electric currents. When it does contract it produces pressures of at most 30 mm. of water, barely enough to overcome the resistance of the valves in the cystic duct. The writer has tried several times to stimulate the gall bladders of animals (anesthetized with urethane and opened under salt solution) into visible activity, but without success. We know also that at operations on men or on animals, the gall bladder is practically never found empty, as it would be if it occasionally squeezed out its contents. The available evidence suggests strongly that bile flows from the gall bladder, not because the muscle there has contracted, but simply because the pressure in the common duct has been lowered by the relaxation of the sphincter at the duodenal end.

Certainly, in the face of these physiological data, the users of the Meltzer-Lyon technic can no longer assume with any confidence that the dark bile comes from the gall bladder. There are a few experiments recorded which suggest that occasionally that may be the case, but most of them show conclusively that the color changes can be obtained in the entire absence of a gall bladder, that is, when that organ has been removed by operation or closed off by disease. It follows that if we cannot safely assume that the dark bile has come from the gall bladder we must not attempt to diagnose cholecystitis because we find bacteria or detritus in that bile. Furthermore, it must be remembered that although the duodenum contains fewer bacteria than does any other part of the bowel, it generally contains some; and it has been shown that an increase in their number will depend largely on the degree of acidity of the gastric juice, upon the amount of saliva swallowed, the sterility of the last food eaten, the condition of the teeth, etc. Another difficulty is that in a surprisingly large proportion of cases of gall bladder disease operated upon, no bacteria can be grown from the bile even with the most advanced cultural methods. Even in the presence of stones, more than 50 per cent of the biles are sterile. Unfortunately, few of the men who have tried to use the Meltzer-Lyon technic diagnostically seem to have been aware of these limitations and sources of error.

THERAPEUTIC PROBLEMS

It seems pretty clear from this review that as a diagnostic procedure the method has hardly a leg to stand upon. Let us see now if it fares any better when we come to examine its therapeutic pretensions. To begin with, we can admit two things: First, that it cannot be expected to have any influence on gall stones; second, that it may easily be of some benefit in the treatment of catarrhal jaundice. Even there, however, it is hard to say how much good it does because the patients get well anyhow, and one can never tell in a given case whether or not the period of illness has been shortened by the treatment. Thus, recently, one of my patients began to fade in about three days. If I had done as I had planned, and had given him one or two Meltzer-Lyon treatments, the outcome in his case would have furnished a strong argument in favor of the new technic.

The great difficulty in treating chronic cholecystitis is that we are faced with an infectious process which is localized not only in the walls of the gall bladder but, as Graham and Judd have shown, is generally scattered through the liver, through the glands about the cystic duct and often into the head of the pancreas. Even if we could clear up this infection with a few treatments, how could we hope to prevent its recurrence; and why should we expect a "non-surgical drainage" to succeed when the surgical drainage has so often failed? As is well known, a large proportion of the people who are relieved of their symptoms after operative drainage, return later for a cholecystectomy, because the infection has returned and new stones have been formed. Meyer, at the Hooper Foundation, has produced chronic recurring cholecystitis in animals simply by putting small foreign bodies into their gall bladders. This little handicap so lowers the resistance of the organ that although it may clear itself of the infection from time to time, it cannot remain clear. Similarly, a woman with gall stones or a scarred gall bladder, is subject to a flareup of infection whenever she has a cold, or whenever she puts undue strain on her digestive tract.

That being the case, it seems to me that there is only one thing to do with a chronically diseased gall bladder if we really want a cure, and that is to take it out. Individual cases will always have to be treated individually, however, and in the present state of our knowledge it is often hard to decide when a patient should be urged to go to the surgeon, and when she should be advised to let well enough alone.

I am glad to see that Lyon now admits that we must be careful how we speak of "cures" in an essentially chronic disease, and that he advises immediate operation in many of the bad cases. I agree with him that his technic is worth trying on certain people who for one reason or another cannot submit to operation, or who have had unsuccessful or partly successful operations. Furthermore, I think it should be tried in every rebellious case of catarrhal jaundice. The method may occasionally be helpful in establishing the presence of severe cholecystitis, but I would never think of relying upon it for the diagnosis of early or obscure forms of the disease.

THE ELECTROGASTROGRAM AND WHAT IT SHOWS *

WALTER C. ALVAREZ, M.D.
SAN FRANCISCO

After several years spent in devising special apparatus and overcoming technical difficulties, I am now getting good records of the action currents in different parts of the stomach and bowels of laboratory animals. The first report of this work has already been published.[1] The records in that article show that the electrograms obtained by leading off from various parts of the stomach and bowel to an exceedingly sensitive d'Arsonval galvanometer resemble closely the kymographic records obtained by attaching light levers to those parts or to bits of muscle excised from them. This resemblance is striking, as regards not only the rate of contraction but also the general form and amplitude of the curve.

We have, then, at our disposal a new method with which to study the activities of the digestive tract. One of its great advantages is that records can now be obtained at all times even from stomachs and bowels which seem to be motionless. Hitherto, physiologists have often been hampered by the visceral paralysis which results from opening the abdomen, but it is seen now that the resultant splanchnic stimulation can abolish the visible contractions without stopping the rhythmic currents. To use a mechanical simile, the underlying chemical processes seem to go on after the nerves have thrown out a sort of "clutch" between them and the contractile substances in the muscle.

THE GASTRIC PACEMAKER

Naturally, the first question to be asked of the new method was: Where is the gastric pacemaker and just

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* From the George Williams Hooper Foundation for Medical Research, and the Department of Medicine, University of California Medical School.

[1] Alvarez, W. C., and Mahoney, L. J.: Am. J. Physiol. **58**: 476-493 (Jan.) 1922.

how do the waves run over the stomach? Although, as often happens, the work has been more fruitful in new puzzles than in answers to the original one, certain observations may briefly be reported at this time.

Previous work had made me feel that the pacemaker would probably be found on the lesser curvature near the cardia, because the muscle excised from that part shows a greater rhythmicity than that of any other part of the stomach. Furthermore, it had seemed to me most probable that the waves which we see arising here and there about the middle of the stomach have really come as ripples from this pacemaker. The electrogastrograms now show clearly that this is often the case, and that definite electrical disturbances are traveling over the fundus when no movement of the muscle can be detected even with a magnifying glass. With suitable apparatus, however, I have been able to show that the muscle does move, usually through distances of only a few thousandths of an inch. Sometimes, as in the irritable stomachs of persons with duodenal ulcers, these waves beginning near the cardia are so deep that we can easily see them with the roentgen ray and without magnifying devices.

DIFFERENT TYPES OF PERISTALSIS

If all the gastric waves began at one point and traveled evenly to the pylorus, the problem would be relatively simple; but, unfortunately, the records, and we already have a hundred yards of them, show all sorts of irregularities. There are shifts of the pacemaker, sometimes even to the pyloric region; there are contractions appearing here and there which do not seem to set up any traveling disturbances; there are waves which travel half way across the stomach only to be blocked suddenly at some point (Fig. 2 *B*); and there are at times systoles of the stomach as a whole. Confusion arises not only on account of the number of these different types of gastric peristalsis but also on account of the sudden changes from one type to the other.

The problem of analyzing the records is complicated still further by the fact that the rate of travel of the waves down the stomach seems to vary markedly from time to time. One set of waves may take seven seconds each to go from the cardia to the antrum, while shortly afterward another set may take twenty-one seconds each. This irregularity, together with the fact that two or three waves are often traveling over the stomach at the same time, makes it very difficult to identify and correlate the deflections which appear in

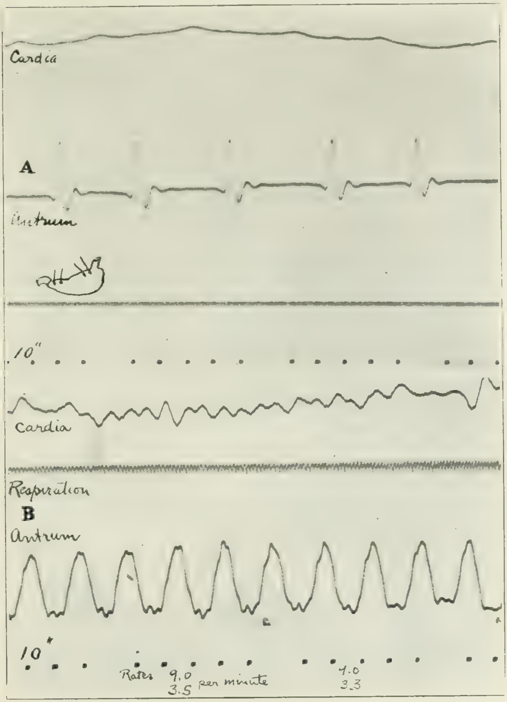

Fig. 1.—*A*, simultaneous electrogastrograms from the excised stomach of a cat placed in a bath of warm oxygenated Locke's solution. The small diagram shows the location of the electrodes. In this and in all subsequent graphs an upward movement indicates a negativity of the orad electrode. Note smaller waves between the main ones on both records. *B*, simultaneous electrogastrograms from the stomach of a cat under urethane anesthesia and with the abdomen opened under warm Locke's solution. There appears to be a complete dissociation between the activities of the two parts of the stomach. The time record represents ten seconds, with a twenty second interval every minute.

records taken simultaneously from different parts of the organ.

DISSOCIATION OF FUNDUS AND PARS PYLORICA

Still more puzzling and surprising is the discovery that the body of the stomach and the pyloric antrum can contract with separate rhythms, much as the dissociated auricle and ventricle do after complete heart-block. I had not been getting electrogastrograms long before I found a number in which the cardiac record showed from three to four times as many deflections as the antral (Fig. 1 *B*). Now, although my previous work had led me to look on the stomach as a combined crop and gizzard, that is, two organs with different functions and different types of muscle, I was not prepared for quite so much dissociation of activity. Furthermore, it had never occurred to me that twelve contractions a minute could appear in a stomach over which three waves a minute are passing. Hence, I felt the need for checking, as soon as possible, the accuracy of the electrogastrogram with simple mechanical methods. That is now being done in my laboratory: simultaneous kymographic records are being obtained from different portions of the stomach, and I can already say that there is no doubt about the dissociation at times between the activities of the fundus and the pyloric antrum, or about the ability of the gastric muscle to respond to two or more rhythms at the same time (Fig. 2 *A*). When we come to think of it, this should not be surprising, because we know that rush waves travel down the small intestine from time to time over muscle which is not only contracting from ten to twenty times a minute, but is responding constantly to marked tonus changes. Incidentally, the work of Palugyay,[2] who screened the stomachs of men and women lying head downward at an angle of 45 degrees, suggests strongly that the small, frequent fundus waves are present sometimes in man.

PYLORIC BLOCK NOT ABSOLUTE

Another interesting point brought out with the new methods is that the stoppage of the gastric waves at the pylorus is not so complete as has hitherto been

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[2] Palugyay, J.: Arch. f. d. ges. Physiol. **187**: 232, 1921.

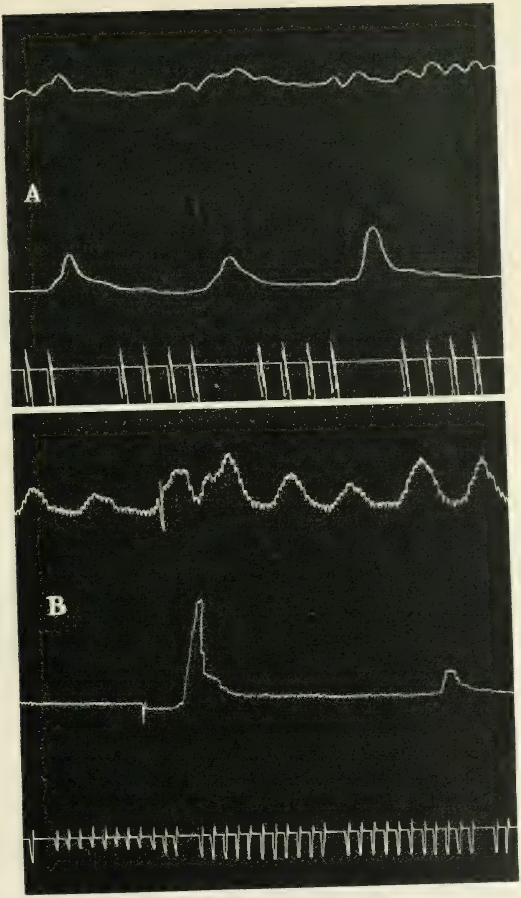

Fig. 2.—*A*, record obtained by fastening light levers to the peritoneal surface of the excised stomach of a dog, kept in warm Locke's solution. Note in the upper, fundus, record small frequent waves superimposed upon the larger ones. The lower record is from the pyloric antrum. *B*, record obtained by fastening delicate enterographs to the peritoneal coat of a sickly rabbit's stomach. The animal was under urethane and had its abdomen opened in a large tank of warm Locke's solution. The upper record is from the preantrum; the lower, from the antrum a centimeter away. Note the block with occasional escape. The small waves on the preantral record are respiratory in origin. The time record represents five seconds.

thought. We can see now that the approach of a wave in the pars pylorica is the signal for the appearance of a strong tonus wave in the first portion of the duodenum (Fig. 3). If this can be shown to occur also in man, we shall take a step forward in explaining the production of the pain in duodenal ulcer.

The new records show, moreover, that the rush waves down the bowel always originate in a gastric contraction which, as we have seen, raises the tone of the duodenum. Furthermore, the needs of the bowel seem often to affect the intensity of gastric peristalsis. Thus, when the duodenum has been contracting actively for some time, trying to send a rush wave down the bowel, the stomach seems often to come to its aid with one or two powerful waves. At other times, a sudden increase of activity in the lower bowel will temporarily stop the gastric waves.

I have long suspected from watching peristalsis in man and animals that some sort of disturbance travels well out ahead of the visible wave, and I can now show it clearly in the records. This observation may have considerable importance because, when the pylorus is a little more irritable than usual, its latent period is likely to be shortened. The muscle there will respond out of its turn, and its contraction will block the main part of the wave before that can bring force to bear on the sphincter. I believe that we can in this way account for the failure of some stomachs to empty properly in spite of good waves and the absence of any visible obstruction at the pylorus.

THE HUMAN ELECTROGASTROGRAM

I had not been working long with the galvanometers before I began experimenting to see what could be done on man. I first proved to my satisfaction that good electrogastrograms could be obtained by applying nonpolarizable electrodes to the shaved abdominal walls of rabbits. The apparatus was then used on a little old woman whose abdominal wall was so thin that her gastric peristalsis was easily visible. This was very convenient, as we could see where to place the electrodes and we could also make a mark on the record every time a wave passed by. A glance at Figure 4 will show that although one of the records, derived presumably from the pars pylorica, has three deflec-

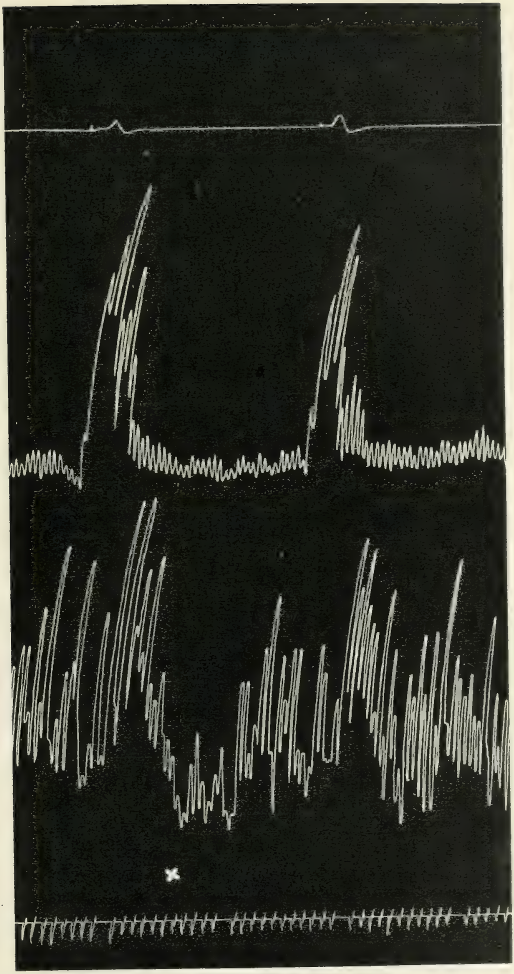

Fig. 3.—Mechanical records obtained by fastening enterographs to the peritoneal surface of the pyloric antrum, first portion of the duodenum, and duodenum 20 cm. below the pylorus. The animal was a rabbit, under urethane, and with the abdomen opened under Locke's solution. Note the large tone changes in the duodenum when gastric waves approach the pylorus. At *x*, the drop in tone shows that a rush wave has just passed on its way down the bowel.

tions a minute, corresponding to the gastric waves, the other one has from eleven to twelve deflections superimposed upon waves corresponding to the visible ones. This record looks so much like many of those obtained from the cardiac region in cats and dogs that I strongly suspect that its deflections are due to small contractions in the upper part of the stomach. My efforts to get similar records from other persons with better muscled abdominal walls have not been so successful. In some, no deflections were seen, while in others only a respiratory record could be obtained. Better results may later be obtained by amplifying the delicate currents.

Some records are now being obtained with an intragastric electrode which can be introduced very much like an Einhorn tube. As modifications are still being made in this instrument, I will reserve its description for a later paper. Suffice it to say that it is a small calomel electrode, 4 mm. in diameter and 2 cm. long. With this apparatus, we can record the gastric contractions of patients with achlorhydria but, as I feared, we have had no success with persons with normal acidities. The voltage produced by slight differences in the concentration of acid bathing the instrument are so great that I doubt very much whether anything can ever be done along these lines.

COMMENT

We see, then, that a study of the action currents of the stomach and bowel has already brought to light a number of interesting things. Still better, perhaps, it has offered us a number of puzzles for solution and has caused us to ask many questions. The future alone will tell us how common the irregular types of peristalsis are in man, and whether they are normal variations or definitely pathologic.

Perhaps the most important service such studies are likely to render the clinician is the demonstration, first, that certain peculiar types of peristalsis exist; secondly, that they are to be looked for under certain conditions; and, thirdly, that they give rise to certain symptoms. To illustrate, we may some day be able to say that the desire to belch is associated with one type of peristalsis; the relief obtained by taking a little soda is due to the institution of another type; the pain of ulcer to yet another type, and so on, just as the heart specialist,

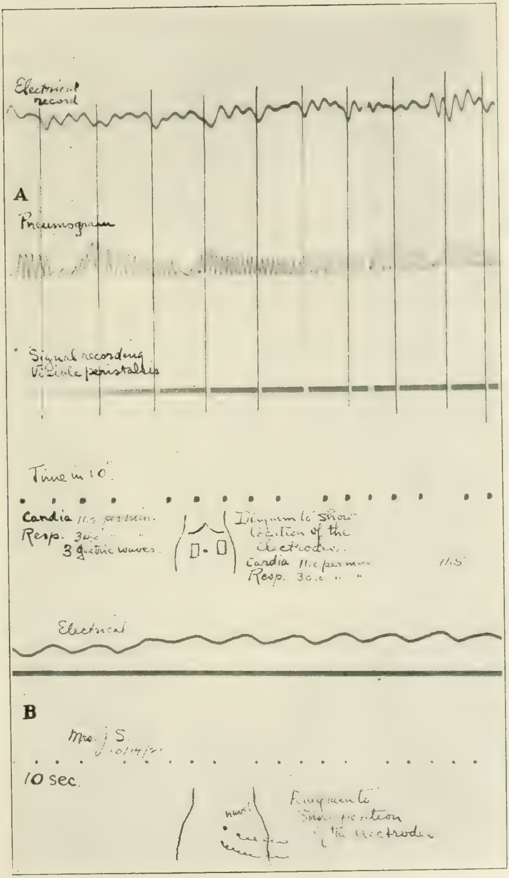

Fig. 4.—Human electrogastrogram. *A,* the small, frequent fundus type of deflection superimposed upon larger deflections which correspond in time with the visible gastric waves. *B,* the antral type of deflection. The small diagrams show the location of the electrodes on the abdomen.

after long experience with the electrocardiograph, can now make shrewd diagnosis of fibrillation, heart block, and so forth, on the basis of the symptoms or the feel of the pulse. Since writing this little prophecy, I have been delighted to see a start made toward its fulfilment in the excellent paper of Reynolds and McClure.[3] They have been screening patients with ulcer for hours at a time, watching to see what happens when pain comes and goes, and they have observed that the onset of such pain often coincides with a change from one type of peristalsis to another. These differences in type have been observed by roentgenographers for years, but no one has taken the time to correlate them with symptoms as Reynolds and McClure have done.

SUMMARY

The first steps have been taken in working out the technic of electrogastrography. The records show that many of the waves which seem to begin in the lower third of the stomach have come as shallow ripples from a pace-making region near the cardia. There are, however, a number of different types of gastric peristalsis, and the stomach can change suddenly from one to another.

At times, there seems to be a dissociation between the activities of the fundus and the pars pylorica. The fundus can contract several times to the antrum's once. Small, frequent waves are sometimes superimposed upon the larger, slower ones. These findings have been confirmed with mechanical recording devices.

Marked blockage of the waves has been observed often in the preantral region.

The conduction time of waves traveling down the stomach shows wide variation.

Marked tonus changes occur in the duodenum coincident with the arrival of gastric waves at the pylorus. This observation may throw light on the production of pain in duodenal ulcer.

The peristaltic rushes down the bowel are shown to originate in gastric waves.

The first human electrogastrograms are here presented.

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[3] Reynolds, Lawrence, and McClure, C. W.: Motor Phenomena Occurring in Normal Stomachs, in the Presence of Peptic Ulcer and Its Pain, as Observed Fluoroscopically, Arch. Int. Med. **29**: 1 (Jan.) 1922.

Reprinted from The Journal of the American Medical Association
April 15, 1922, Vol. 78, pp. 1116-1119

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Reprint from the California State Journal of Medicine,
September, 1922.

THE DIAGNOSIS AND TREATMENT OF GALL BLADDER DISEASE.*

By WALTER C. ALVAREZ, M. D.

PART II.

The first installment of my article, published last month, dealt largely with what not to do in the diagnosis and treatment of gall bladder disease; this second part will deal briefly with the things which I think should be done.

THE FREQUENCY OF GALL BLADDER DISEASE

First, I should state that statistics from many sources show that from five to twelve per cent of all women coming to autopsy have gall stones. If we exclude women under fifty, the percentage is still higher. This alone would be bad enough, but we know that there must be a still larger group of men and women in the community suffering from the pre-calculous stages of cholecystitis. There would seem, then, to be no escape from the conclusion that even the careful clinician is not making the diagnosis of gall bladder disease often enough. The writer gets the idea sometimes that he is becoming mentally warped on the subject because he suspects cholecystitis in every old lady who comes in with flatulence and abdominal pain; and then along come a few autopsies or operations on his patients and he discovers a number of gall stones, the presence of which he had not even suspected.

Now, how are we going to recognize a larger percentage of these cases?

A GOOD HISTORY

First, and above everything, comes a good history. A case will often seem puzzling until skillful questioning brings out the story of one or more attacks of supposed ptomaine poisoning which really had all the earmarks of gall stone colic. The physician must remember that recurrent attacks of severe pain which perhaps waken the patient out of a sound sleep and which leave her upper abdomen sore for days afterwards are due to organic disease; they cannot be ascribed to a neurosis or to slight indiscretions in diet. In the most typical cases the pain begins in the liver region and runs up into the right shoulder blade. Often there is a constant ache on the right side. The soreness is aggravated by riding over a rough road or by reaching up on a shelf. Women perhaps will be unable to bear the pressure of their corsets in the liver region. In milder cases and in the intervals between attacks, the patients complain mainly of belching and bloating. They often feel "bilious" and get a little sallow. Only rarely are they jaundiced. Nausea, regurgitation of food and heartburn are common. Vomiting often comes during the attacks of pain.

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* Read before the Section on General Medicine of the Medical Society of California May 16, 1922 at Yosemite National Park.

Most characteristic and helpful is the patient's statement that although she has a fine appetite she is afraid to eat. She dreads the return of pain. Yet she will admit that what she eats seems to have little or nothing to do with the coming of an attack. She may get a bad one after a week of fasting, and later she may digest a Thanksgiving dinner without discomfort.

Several suggestive points may be mentioned. A history of a useless appendectomy shows that the symptoms were so bad that the patient was willing to do anything to get well. A history of typhoid fever, chronic sinusitis, empyema, or any other severe and prolonged infectious process is helpful because such things often leave the gall bladder damaged irreparably.

The presence of arthritis, myocarditis and certain forms of headache and dizziness is suggestive because these troubles sometimes clear up after cholecystectomy.

THE PHYSICAL EXAMINATION

This generally shows a woman between forty and sixty-five although, as our experience grows, we are going to recognize cholecystitis more and more in the twenties and thirties, and even in childhood when the trouble often begins. In subacute cases the patient gives a jump when a deep breath brings the tender liver edge down against the palpating hand (positive Murphy's maneuver). The presence of fibroids in the uterus, or other pelvic abnormalities, greatly increases the probability of finding gall bladder disease.

ROENTGEN-RAY EXAMINATION

This can help greatly in several ways. First, it may exclude the presence of other organic lesions such as ulcers and carcinomas.. Second, it may show the shadow of stones or of a thickened gall bladder on the plate. Unfortunately most of the diseased gall bladders are free from stones and so thin-walled that they will not show on the plate. Furthermore, many stones are too soft to give a shadow. Third, the screen examination often shows many suggestive things. Cardiospasm is often seen, and I am coming to think more and more of it as an indicator of gall bladder disease. The stomach is often hypertonic and overly active; and not infrequently the pyloric antrum is contracted and sharply conical in shape. There may be some gastric stasis, and it may also be apparent to an experienced observer that the gastric mucosa is dry (achyliac). The duodenal cap is sometimes deformed by adhesions or pressure, and it may also show defects in emptying. The colonic haustration is often exaggerated. The liver is not infrequently enlarged from the cirrhosis attendant upon prolonged infection of the bile ducts.

LABORATORY TESTS

Gastric analysis often shows an achlorhydria. In the first part of my paper I have explained why I think the Meltzer-Lyon test should not be used diagnostically.

TREATMENT

Like appendicitis, gall bladder disease may go on and kill the patient or it may clear up even without treatment. Once cleared up, it may leave the victim alone or it may come back at intervals until finally something has to be done. We know now that it is best and safest to take diseased appendices out. Unfortunately, we do not know yet what to do with the early cases of gall bladder disease. If we could only look into the future for the different individuals it would be easy. When I see women about sixty-five, emaciated, worn with suffering, anxious for an operation which they cannot well stand, I tell myself that it is my moral duty to urge the younger ones to go to the surgeon. When, however, I see a series of women about forty who have gone ten or fifteen years without attacks or much distress, I waver, and am inclined, as is the patient, to let well enough alone.

Unfortunately, it seems impossible to say when these people are really "cured" and free from the menace of their diseased bile tracts. Thus, one would think, after several attacks of gall stone colic that an interval of twenty years without troublesome symptoms would indicate a cure, but I have seen a woman in whom such a period of relief was really only an interlude. When she was sixty-one her troubles returned; a plate showed that a stone still remained, and an operation showed that a carcinoma had developed somewhere in the biliary tract.

It is hard to say whether medical treatment does much good because remissions occur so frequently and come so suddenly. Ordinarily it is well to advise a smooth, cellulose-poor diet; that is, without salads, coarse vegetables, or fruits. Some people are helped by the taking of Carlsbad salts in hot water before breakfast. Belching and heartburn are often helped by the giving of sodium bicarbonate and magnesia. Hoffman's anodyne often brings relief during attacks at night. During paroxysms of pain, paregoric, morphine and papaverin have to be used. The physician should remind the patients who are losing weight that they will probably have trouble whether they eat or not, so that they had better eat and keep their strength.

DIFFERENT TYPES

For therapeutic purposes the cases may at present be divided into six or seven groups. Medical treatment should be reserved for:

First, those who are in the early stages of the disease, with mild symptoms and long intervals of relief, whose bodies are still young and strong, and who may perhaps yet hope permanently to overcome the infection.

Second, those whose symptoms and findings strongly suggest the presence of cholecystitis but who are suffering so much from nervousness, menopausal storms, hypertension, or myocarditis that it is a question whether they would be any better off even if a markedly diseased gall bladder could be removed safely. These people must be studied carefully before any decision is made in regard to operation.

Third, those who need and want an operation but who, on account of complications, cannot undergo it with any prospect of success.

Fourth, those who have had a cholecystectomy but who still suffer. Many of these people might have gotten a perfect result if they had been put on a smooth diet after operation.

In the absence of definite contra-indications operation should be advised for:

First, those who have reached that stage of the disease in which the attacks are getting steadily worse and more frequent.

Second, those whose indigestion is severe enough to interfere with ability to work and to enjoy life. They should be the more willing to accept operation if they have an arthritis, headaches, dizziness or myocarditis, which may perhaps be cured by the removal of the infected gall bladder.

Third, those who have had their gall bladders drained and who had gotten relief, but who now return with pain and perhaps with new stones.

Finally, and this is a most important point: The surgeon must be willing to admit that at the time of operation he cannot tell a diseased from a normal gall bladder. Men of experience know that not infrequently gall bladders which, according to the history, must have been discharging stones off and on for twenty years, are found at operation to be still soft and apparently normal. If they were not full of stones the surgeon would generally pass them by as inocuous. This does not mean that I am arguing for the reckless removal of gall bladders. Far be it from me; but as Judd says, there is no doubt that in many cases if the patient is to be helped, his gall bladder must be removed on the strength of a careful history taken by an experienced man. This removal can be done with more confidence if there are no other signs of disease in the abdomen to explain the severe symptoms, and if there are adhesions about the gall bladder, an enlarged gland near the cystic duct, an enlarged liver and signs of perihepatitis and pancreatitis. I am convinced that the poor results obtained in many of the cases of operative interference for duodenal ulcer can be ascribed to the leaving behind of gall bladders which had become badly involved in the inflammatory process about the bowel.

IMPROVEMENTS IN PREOPERATIVE AND POSTOPERATIVE CARE *

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AND
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Until a few years ago, practically every patient who was to undergo a serious operation had to submit to a course of violent purgation. Many of the older surgeons were partial to 10 grains of calomel with 10 grains of jalap, while others gave castor oil and salts in large doses. On the morning of the operation, enemas were given one after another until the water returned fairly clear. It mattered not whether the operation was a colectomy or a tonsillectomy, the patient was "prepared" just the same. For many years, however, the tendency among the more thoughtful surgeons was to cut down on the severity of this preliminary purgation, and today many men are using enemas alone or else mild laxatives, given thirty-six hours before the operation so that the patient can recover from their disturbing effects. Many surgeons have given up the routine purgation because they have seen cases in which it unquestionably did great harm; others have done so because they noticed that their unprepared emergency cases did better than their well "prepared" ones.

In 1917, Alvarez and Taylor[1] made a physiologic study of the bowels of rabbits purged with various drugs, and found that the intestinal muscle after catharsis is toxic, weakened and erratic in its responses to stimuli. The circulation is upset, and with it the delicate mechanism which regulates the absorption of

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[1] Alvarez, W. C., and Taylor, F. B.: Changes in the Rhythmicity, Irritability and Tone in the Purged Intestine, J. Pharmacol. & Exper. Therap. **10**: 365 (Nov.) 1917.

gases from the lumen of the bowel. These studies offered an explanation for the well-known malaise and flatulence experienced by many persons for a few days after purgation. They certainly offered no support to the idea that purging is a suitable preparation for the ordeal of having the abdomen opened.

In a subsequent paper, Alvarez [2] reviewed the history of the subject and showed that preoperative purgation is a relic or lineal descendent of Greek surgery and humoral pathology. The original idea was that "black bile" was responsible for the disease to be eradicated, and that it was useless to operate until the supposed cause had been purged away. He pointed also to the fact, well known to roentgenologists, that the small intestine practically always empties itself within a few hours after eating, and that the large bowel can in most cases be emptied easily and completely by enemas. He showed, moreover, that what the surgeon should desire above all things is not an empty bowel but a bowel with a normal circulation that will carry off any gas that happens to be formed. Every effort should be made also to preserve the normal downward gradient of muscular forces so that food and gas will go toward the anus and not back toward the mouth. Alvarez argued against the *routine* use of purgatives, and suggested that they be given only when distinctly indicated. Shortly afterward, Peet,[3] Emge,[4] and Wilensky [5] presented similar arguments, strongly urging the abandonment of preoperative purgation. These papers encouraged many men to give up the practice altogether, and others were induced to lessen the severity of their "preparation," so that, today, so far as we can learn, there are very few surgeons who cling to the old technic.

Until 1919, it was customary at the University of California Hospital to give a dose of castor oil the night before operation, and enemas in the morning. At that time, Dr. Terry discontinued the routine purgation, but left the order for enemas. The general impression about the hospital since then has been that the postoperative recoveries are on the average more satisfactory, and that there is less vomiting, abdominal distention and pain. Several of the operators are enthusiastic over the change, and no one has expressed any desire to return to the old routine. In 1918, Alvarez [2] suggested that it would be wise also to abolish the routine purge usually given on the second or third day after operation. Some of the nurses had remarked upon the fact that not infrequently everything went well until that purge was given, and then everything went wrong. It seemed often to give rise to the vomiting, gas pains and tympanites; or, if they had been present before, it seemed to bring them back. Moreover, there does not seem to be any good reason for giving purgatives to these patients so soon after operation. One cannot expect them to produce fecal material when, for several days, they have had little or no food. The main reason for giving this purge is that, ordinarily, the surgeon feels uneasy about his

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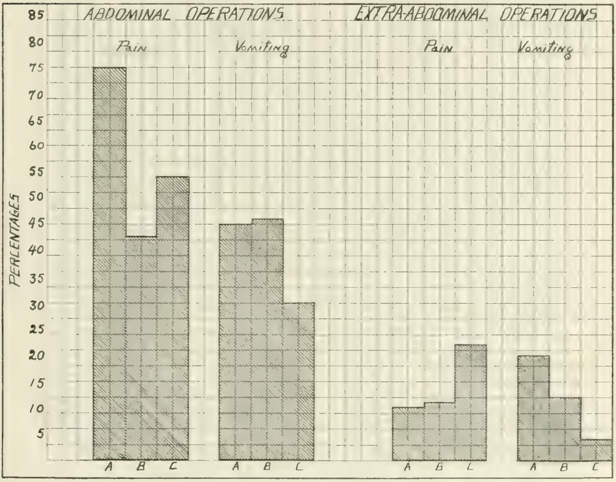
[2] Alvarez, W. C.: Is the Purgation of Patients Before Operation Justifiable? Surg., Gynec. & Obst. **26**: 651 (June) 1918.

[3] Peet, M. M.: Rational Preoperative Treatment, with Special Reference to Purgation, J. A. M. A. **71**: 175 (July 20) 1918.

[4] Emge, L. A.: The Prevention of Gas Pains, J. A. M. A. **71**: 878 (Sept 14) 1918.

[5] Wilensky, A. O.: Prog. Med., June, 1920, p. 70.



Incidence of pain and vomiting in patients: *A*, purged before and soon after operation; *B*, not purged before, but purged soon after operation; *C*, not purged before and not purged for four days after operation.

patient until the bowels begin to move. After that, he feels that the worst dangers are over; but, as the purge can do harm and can cause much discomfort, it would undoubtedly be better sometimes if the operator would restrain himself a bit and wait patiently for Nature.

As some of our surgeons felt a certain amount of uneasiness about giving up such a long-established custom, it was thought wise to observe the results of the new technic very carefully and to compare them statistically with those obtained in the preceding period when patients were purged before and after operation.

An effort was made to answer the following two questions: (1) Has it been advantageous to give up the routine preoperative purgation; and (2) has it been advantageous to give up the routine postoperative purgation?

Sixty-two of the people whose postoperative records have been analyzed in this paper were watched and questioned day by day by one of us (F. B. T.). Unfortunately, our conclusions in regard to the control group operated on before 1919 must be based solely on the nurses' records. Most of these are from the University of California Hospital; some are from Lane Hospital, some from the Children's Hospital and some from Mount Zion Hospital. Unfortunately, nurses are often kept pretty busy, and we cannot be sure that every complaint of pain and every attack of vomiting has been charted. However, on comparing the nurses' records in the last sixty-two cases with the notes made independently by Dr. Taylor, we feel that the nurses can be trusted to give a fair idea of the storminess or mildness of a postoperative period. One point in favor of the hospital records is that they were made without bias, i. e., the nurses had no idea of the use to which their notes were to be put. In analyzing these data, no attention has been paid to the patients' symptoms on the day of operation because we must expect some suffering at that time from the effects of the anesthetic and of the incision.

Our conclusions are based on records from 211 patients. Of these, 146 had abdominal, and sixty-five had extra-abdominal operations. The abdominal series includes appendectomies, hernioplasties, gastroenterostomies, cholecystectomies and explorations. As it would obviously not be fair to compare the postoperative symptoms after partial gastrectomies with those after simple hernioplasties, an effort has been made to have the different types of operations represented about equally in the control and the experimental groups. Aside from this, the cases were taken serially, without any selection or discrimination. The extra-abdominal group includes most of the common types of operation performed in a large hospital.

In the analysis, the cases have been divided first into abdominal and extra-abdominal. Naturally, we would expect the patients who have their abdomens opened to suffer more from gas pains and vomiting than do those who do not have them opened. Each of these groups is divided again into three: (1) those operated on before 1919, who had been purged before and after operation; (2) those who had not been purged before but had been purged shortly afterward, and (3) those who had not been purged before and who were given no enema or cathartic for at least four days after operation. The incidence of pain and vomiting in these three groups is shown graphically in the accompanying chart.

The first thing of interest to be noted in the chart is the fact that even the patients who did not have their abdomens opened sometimes had considerable pain and even some vomiting. It shows the large influence of the anesthetic in the causation of these postoperative disturbances.

The salient points brought out are: (1) The omission of the preoperative purge had no definite influence on the amount of vomiting, but it had a decided effect on the amount of pain in the abdominal cases, reducing its incidence from 75 to 42 per cent.; (2) the delay in giving the postoperative purge had a decided effect on the amount of vomiting, reducing its incidence from 45 to 30 per cent. in the abdominal group, and from 20 to 4 per cent. in the extra-abdominal group; (3) in both groups, there was more complaint of pain when the postoperative purges and enemas were withheld. We believe that this means that a number of persons will be more comfortable if given enemas as soon as they are needed to help in the passage of gas. The essential thing is to make the treatment individual and not routine. We still feel it wise, however, in most cases to avoid the use of cathartics for as long a time after operation as possible.

Unfortunately, in going over the records no account was kept of the severity or duration of the vomiting and pain in the various groups. Our clinical impressions lead us to believe that such data might have shown the improved results more graphically than do those which we present here. When one comes to think of it, it is really rather remarkable that 55 per cent. of persons who have within a period of a few days submitted to purgation, to an extensive laparotomy and then to purgation again should (after the first day, as shown above) escape vomiting, and that 25 per cent. should escape abdominal pain. Another 20 per cent. are so constituted, however, that they will have gas pains and vomiting after minor extra-abdominal operations, with no purgation and only a short gas-oxygen anesthesia. The importance of this large personal element is seen in the case of a woman who recently had the gallbladder removed. She had no purgation, and the anesthetic was gas-oxygen. She had no gas or vomiting, and ate with pleasure on the day after operation. At first sight it would seem to be a triumph for the new technic; but it appears that she did just as well seven years ago, when she was well purged and when she had an ether anesthesia for the drainage of the gallbladder.

One point is gratefully noted by the nurses, and that is that they have much less work to do under the present routine. There is less carrying of bed pans; there is less moving of people in heavy casts, and there is less soiling of patients and beds.

As some surgeons have worried a bit about the dangers of fecal impaction or autointoxication, twenty patients were allowed to go for at least six days after operation without receiving purges or enemas. None of these patients showed any ill effects, and several of them had spontaneous bowel movements within the period of observation. In the others, an enema on the sixth day gave good results. Certainly it would seem that a surgeon need have no fear about neglecting his patients' bowels after operation, particularly when little or no food has been given out of which feces can be formed.

The essential point which we wish to make is that in a large hospital with young and inexperienced house officers, the standing orders should call for as little meddlesome interference as possible. If any patient develops symptoms which require the use of purges or enemas, they can always be prescribed; but they should not be made compulsory for the patient who is convalescing smoothly and rapidly. Furthermore, the standing orders must not be such that the very life of a man coming in with an acute intestinal obstruction, a perforating appendix or a gangrenous Meckel's diverticulum must depend on the intervention of some intelligent intern or resident.

Some may feel that our figures do not show enough improvement with the new technic to warrant spending much thought on it. Our answer is that until a few years ago a man might be satisfied if he escaped from an operation with his life and some improvement in his condition. Today, operations have been made remarkably safe. The next thing is to reduce the amount of discomfort attendant on them. If, by any procedure, surgeons can eliminate postoperative vomiting in fifteen out of a hundred cases, and postoperative pain in thirty-two out of a hundred, they will be doing something worth while, something which will redound to their credit individually and collectively.

Reprinted from the Journal of the American Medical Association
Nov. 4, 1922, Vol. 79, pp. 1578 and 1579

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Mutations in the Genus Nicolaierillus (B. Tetani)

STUDIES ON PATHOGENIC ANAEROBES VIII

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Reprinted from
The Journal of Infectious Diseases, Vol. 30, No. 1, Jan., 1922, pp. 33-49

MUTATIONS IN THE GENUS NICOLAIERILLUS (B. TETANI)

STUDIES ON PATHOGENIC ANAEROBES VIII

From the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco

HILDA HEMPL HELLER

In a former paper,[1] it was suggested that in the study of anaerobic bacilli colony formation in deep agar was available for two purposes: for the differentiation of strains that behaved alike on ordinary mediums, and for the study of mutations. The objection will be made that if anaerobic colonies mutate, they are useless for systematic purposes. This objection holds good for the groups in which mutations occur readily and not for those in which they are rare. No other group studied in this laboratory shows such a wide range of colony types as does the genus Nicolaierillus[2] (B. tetani). The diversity of these colonies has been pictured in a preceding communication.[3] Mutations in deep liver agar are frequent in this proteolytic genus, while among nonproteolytic organisms, although mutations in colony form probably occur, they have not been noted. Moreover, in some genera it has been possible to correlate the different colony types with other characters that may be termed specific, while in the tetanus group it has thus far been impossible to do so. A study of a few mutations in the genus Nicolaierillus is herewith described. Apparently these organisms furnish a most excellent field for the close study of bacterial mutation—here only superficially investigated.

TECHNIC

The technic employed in this study has been described before.[1, 4] It is principally the ancient procedure of Liborius.[5] Inoculum was thoroly mixed in the first of three dilution tubes of deep agar. From this tube a suitable amount was removed with a Pasteur pipet to a second tube, this amount depending on the number of organisms in the original inoculum. The second tube was mixed and a larger amount (0.25-0.75) taken from it and placed in a third. Three tubes usually suffice to give a satisfactory picture of the flora

Received for publication Aug. 18, 1921.

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[1] J. Infect. Dis., 1922, 30, p. 1.

[2] Heller, H. H.: J. Bacteriol., 1922, 7, Jan.

[3] J. Infect. Dis., 1922, 30, p. 18.

[4] J. Bacteriol., 1921, 6, p. 445.

[5] Ztschr. f. Hyg. u. Infectionskrankh., 1886, 1, p. 115.

of an inoculum. In case more chance for mutation is desired, two or three number 2 tubes may be inoculated. A third dilution tube should always be used to give a chance for obtaining in one tube a pure culture of the predominant biotype.

The shaft of agar from which inoculation is desired is blown from its tube into a Petri dish by means of a large-bore Pasteur pipet. The same pipet, with careful flaming, may be employed many times with no chance of contamination. The location of a desirable colony may be marked by the pipet groove in the agar and by a mark on the glass tube to indicate the distance of the colony from the end of the tube. Small colonies may be fished under the binocular or ordinary microscope but such procedure is seldom necessary for tetanus colonies. Fishing is accomplished with the pipet that is to be used for diluting the agar in the next three tubes. The glass is drawn to a needle point, broken and flamed, and inserted into the agar shaft to the desired colony, which is sucked up and transferred to the first dilution tube of a new series. Agar may be cut with a flamed (not a boiled) razor blade or knife; it is unnecessary to "sear" the agar, which process only melts it anyway.

THE HISTORY OF STRAIN T 087

The culture T 087 was received from Parke, Davis and Co. and is one of their regular toxin-producing strains. When first studied, its colonies in deep liver peptone agar were uniform, small, subspherical, with an irregular outline and no long woolly protuberances (plate I upper left figures). After some months of cultivation on meat medium the strain was thought to be contaminated with an organism forming woolly colonies. The small colony type was isolated but contamination by a woolly colony was again observed, and repeated shakings in agar dilutions showed that the supposed contamination must have been a mutation. The history of the behavior of this strain is here given in detail.

The original agar culture (Nov. 4, 1919) was inoculated onto meat and therefrom onto agar dilution tubes. One colony form was observed, the small type mentioned in the foregoing. A colony of this sort passed through a meat culture and was inoculated onto agar again. Two types of colonies were noted; a small one was fished onto meat, and this culture had had 5 passages on meat when, 9 months later, it was reinoculated onto agar, and showed 2 types of colonies in about equal numbers. The larger of these (TOL, plate 1) was replanted many times on agar of the same batch and no reversion or mutation was noted.

A small colony gave in dilution tubes both types, the small being most abundant. The third tube had 6 colonies of each type. The offspring of 2 large ones from this tube were all fluffy like TOL, with which they were probably identical. One small colony gave, in a series of 4 dilution tubes, only one woolly colony (TO 2), which may have been a contamination by TOL, but by its composition was probably a mutant. Its subsequent history was of interest. This one colony gave two types. In the second tube 58% were large. In the third tube 4, which were carelessly abandoned, were large, one was small, one smooth and larger. The story of the smooth larger colony on reinoculation onto agar tubes follows:

1. Very abundant colonies, 2 types.
2. Large, dense, and smooth, 390; small 96.
3. None. ↓

Large dense smooth type one colony onto agar.

1. ?
2. Eighty-nine large, fluffy; 2 small.
3. Two fluffy (TOM, Plate 1).

The smooth large colony, intermediate type between TOM and TO 2 was, sad to say, not photographed, and it is a question whether it may have been the same as TOM or not.

A small colony from TO 2's first subculture, which represented the original type, gave in dilution tubes uniform small colonies of which a single one was markedly asymmetrical, having a woolly side and a smaller smooth side. Inoculation (fishing was done under the binocular) from the woolly half of this asymmetrical colony gave pure dense woolly colonies (TDW, plate 1). Inoculation from the smooth half gave in the third tube 40 dense woolly colonies and 44 smooth ones.

A smooth colony from a sister tube to that from which TO 2 was derived, a tube in which no woolly colonies existed, gave two types of colonies, 41% of them large. As I was at the time still under the impression that these large mutating colonies were all alike, this mutant was not photographed and was lost. Here is an instance, one of several in this series, in which a colony fished at random, had within it mutants that had caused no change in the shape of that colony. It is better to choose for the demonstration of mutation colonies in tubes that contain no mutant colony, so that the question of contamination by permeating growth is eliminated.

To return to the early tube in which there were 6 small colonies and 6 woolly ones: Another colony showed either contamination with TOL or mutation. Twenty-five % of the colonies were fluffy and the rest smooth. One small colony from this generation gave 0.43% large colonies, another 2.9% large ones. These also probably represent contamination by the other type (TOL). One tube of this series contained 100 smooth colonies and no woolly ones at all. When one such colony was inoculated into agar tubes the second dilution tube showed a mutant, one colony in 250, radiate in structure, which type (TRW) in culture tubes did not resemble TOM so closely as it does in the photograph. The third tube contained only 2 small colonies, no woolly ones. One of these on inoculation into agar showed only one type (small) in the first tube, one type in the second (300 colonies), but in the third dilution tube were 12 small colonies and one woolly mutant (TLW), that was larger than all the others, which was easily isolated in the dilution series on which it was planted.

About as many transplants were made of smooth colonies that gave rise to no mutants as of those that did. Their history is omitted.

The photographs on the plate were made under identical conditions at the end of the experiment.

Three-day meat cultures of these 6 strains (T 087, TDW, TOL, TOM, TRW and TLW) were inoculated subcutaneously into guinea-pigs in 0.75 c c doses. The animals showed no symptoms of tetanus. Ten-day meat cultures gave a like result, and 7-day cultures in glucose-veal toxin broth containing blood inoculated on a third series of guinea-pigs caused no symptoms of tetanus. The behavior of all the T 087 strains was culturally entirely typical of that of tetanus bacilli[3] and strain T 087 had long been known as a standard toxin producing strain. Miss P. Schoenholz was so kind as to investigate the agglutination reactions of the organisms and found that all 6 substrains were agglutinated by serums made from strain TT (Tulloch's type 1) in titers from 1000-6000, strain TE (T 089 of the U. S. Public Health laboratory) in titers from 4000-20000 and strain T 087 (a serum that was made shortly after the arrival of the culture in this laboratory) from 4000-8000. It must be concluded that during the frequent fishing of the small type before the commencement of this experiment a wholly nontoxic mutant had been selected from among the toxin-producing colonies.

We have here a record of 4, or 5 or 6 different mutant types, all but one occurring during the 2 weeks' study of a tetanus strain. Apparently this strain mutates in so many directions that the classic demand that to prove bacterial mutation the mutant must be repeatedly found, cannot be satisfied without long search. Had the colonies not been photographed they would have been thought to be alike, and the requirement would have been technically, apparently, fulfilled. No contamination, either by other anaerobes than tetanus, or by aerobes, was noted during the whole study—and such contamination was feared and rather expected, for the laboratory is full of many kinds of anaerobes. Practically every unusual colony was fished and its behavior studied.

It was noted that if the colonies were fished when 24 hours old, mutants were hard to find, but if they were 48 hours old, in a large series of tubes they were usually to be found. Therefore, old material was investigated in order to find whether many mutant forms existed therein.

THE BEHAVIOR OF STRAIN T USA

This strain was furnished by Miss Muriel Robertson who, I believe, isolated it from a stock American strain by means of the Barber technic[6] during the tetanus investigation. She states that with it Tulloch made the serum with which his type 1 strains were identified.[7] It was received in the form of an egg broth culture in the fall of 1919 and bore the designation T USA II and the date September 6. Previously another culture of what was probably the identical strain had been received from Dr. McCoy of the United States Public Health Laboratory. This (TT) was labeled "Tulloch, Type I" and made large fluffy colonies without an opaque center and, more rarely, large opaque woolly colonies (Fig. 2, top row).

In March, 1920, the egg broth culture "T USA II" was inoculated onto meat and therefrom onto agar. Two types of colonies were observed. One was large and fluffy without an opaque center. The other was slightly smaller and was opaque. After isolation, these were photographed (Plate 2, second row), but already after only two successive transplants the original type of clear fluffy colony had been partly replaced by a colony with a small opaque center—T USA W. Both these types were typical tetanus bacilli and later formed high titer toxins that produced typical tetanus symptoms in mice during an immunity experiment.

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[6] Phil. J. Sc., B., 1914, 9, p. 307.

[7] J. Roy. Army Med. Corps, 1917, 29, p. 631; Proc. Roy. Soc., B., 1919, 90, p. 145.

In October, 1920, a meat culture was again made from the original egg broth tube. On agar its colonies were of only one type, large and fluffy. A sister meat culture was sent to Dr. Ivan C. Hall, who complained that it contained two kinds of tetanus colonies, an opaque one that behaved normally and a fluffy one that could not be purified, the opaque type continually reappearing therein.

In April, 1921, the meat culture made in October, 1920, was planted directly into new agar dilution tubes. Instead of one single fluffy type, such as was found in such a transplant in October, a galaxy of divergent types appeared. From the second dilution tube a number of these were fished into a new series of agar tubes and were isolated in pure culture (Plate 2, large group, all but T USA LC). Some of these were alike but between others of the strains were marked differences. The original fluffy type was, apparently, lost. Certain of the colonies were similar when observed with a hand lens, but differences could be observed between them when they were photographed. Such were T USA M and P. Others can be correlated by photographic procedure (USA X and P). T USA F is a larger form than the original type. T USA LC, the only strain pictured that was not isolated from the second dilution tube from which the others originated, is an independent derivative from the original stock, which was a fluffy clear centered strain, and it resembles F in contour but not in size. T USA O was consistently larger than T USA X and P, which are probably the same sort of mutant as the original T USA S. T USA B is distinctly smaller and more smoothly contoured than any T USA S-like colony observed. And, finally, because the parent tube had lain for some days before it was fished, a successful subculture was obtained of a very minute form, T USA T, which is hardly to be discerned with a hand lens after 24 hours' growth, but forms small spherical colonies when 48 hours old.

Thus we see that a tetanus strain, left long on a medium, like egg, that furnishes little nutriment, retains its type, probably through failure to multiply. But, if it be sown on a well-buffered highly nutrient medium, like meat, it will continue to multiply, in so doing it may mutate, and the mutant forms complete, some dying out, some sporulating and waiting for a better day, others increasing, till after a few months, if mutation be active, the culture tube may contain a multitude of biotypes, some better able to multiply on another substratum like agar, and some less able to multiply on it, and some multiplying as abundantly as the parent type but taking, in colonies, different forms.

These strains—T USA-F, M, LC, O, X, P, and T—were all typical tetanus bacilli; 3-day old meat cultures of them when inoculated subcutaneously into guinea-pigs in doses of 0.75 c c killed the animals with symptoms of tetanus inside of 48 hours.

THE HISTORY OF THE STRAIN TG

This strain was isolated from gelatine at the United States Public Health Laboratory from which I received it in an agar stab in November, 1918. It was planted onto meat on November 6, therefrom onto meat June 25, 1919. July 2, agar shakes from this meat culture gave colonies that were invisible in 24 hours, and are shown at that age magnified 50 diameters in the photographs (Plate 3, top left). Colonies incubated longer than 24 hours grew apace and when 5 days old were as in the series below. A smooth colony was fished from this tube and after passage on meat gave colonies in agar that were denominated "irregular, smooth and minute." On July 14, the meat culture that was planted June 25 was again inoculated onto agar tubes and the result was small irregular colonies, at 24 hours indistinguishable, at 48 hours definitely of two sorts—lenticular and woolly. Subsequent transplants were made from the stock termed "irregular, smooth and minute" in September, and the first agar shake showed small irregular compound lenticular colonies (Plate 3, upper right) that, when 3 days old, were not to be distinguished from one another, but when 5 days old were of 2 types, some loose and woolly, some smooth and lenticular, and 2 with bushy protuberances. Both types were fished, but, sad to say, the smooth type was lost and has never been recovered. The strain now is a woolly one (the 4 colonies below) with an enormous range in colony size that is exceedingly baffling to work with. Three quarters c c of 3-day old meat culture inoculated subcutaneously into a guinea-pig caused the death of the animal in a few hours.

It must be borne in mind that this work was done before the significance of these mutations was comprehended, in order to be sure the strain was pure, and in order to take photographs. Whether the differences seen between the two top groups of figures on plate 3 represents a mutation it is now impossible to say. It is probable that it does. But that the woolly type, now the only one preserved, is a mutation from the smaller type cannot be doubted. Of interest is the fact that when crowded the colonies resemble closely their ancestral form. In crowded tubes these compound lenticular colonies are permanent and do not turn woolly except when the agar has not been thoroughly mixed and there is abundant nutriment for the colonies at the edge of the culture. But no pure lenticular form can be isolated from them, for as soon as they are planted in sufficient dilution lenticular forms are almost impossible to find, practically all the colonies being woolly. These woolly colonies vary greatly in size, but whatever sized colony may be chosen for inoculation into dilution tubes the result is always the same—the colonies vary from minute ones to large fluffs with a clear zone about the center.

STRAIN TL

This was received from Dr. S. H. Gilliland and was apparently a pure biotype that made a transparent colony with a tendency to form a thickened cortical layer (Plate 5, top left). Two years later a meat culture of the strain showed heavy opaque colonies of about the same size as the older ones (TLC, second row, left). Search revealed a few transparent colonies among them. After 2 transplants, the transparent type (TLB, top row, right) was recovered and it was impossible to demonstrate whether or not it was identical with the original type. Both of these strains (TLC and TLB) were highly pathogenic for guinea-pigs, causing marked asymmetry and rigidity of the body followed by the death of the animals inside of 48 hours.

It is of interest that these colonies showed a very small range of variation in size. The strain TG in its woolly form shows similar colonies that are a little more irregular in contour than those of TL, but they have a very wide range of variation in size. Strain TP ("Smith, from Pease" sent me by Dr. G. W. McCoy) forms more opaque colonies with a great range in size, utterly unsuited to the study of mutations.

STRAIN TVP

Strain TVP was isolated from an ivory vaccine point by Miss Bengtson and sent to me by Dr. G. W. McCoy. It forms the largest colonies in the whole collection, which is of interest from an epidemiologic point of view when one considers its origin. On first transplantation 2 kinds of colonies were noted. A loose fluffy type was isolated, and gave rise to colonies somewhat inconsistent in behavior, shown in Fig. 4, top and center. Two years later, 2 types, TVPF and TVPD which is derived from TVPF, were discovered in the original tube. What relationships these bear to the colonies pictured earlier is not clear. Three-day meat cultures of TVPF and TVPD killed guinea-pigs in doses of 0.75 c c with symptoms of tetanus in 24 and 48 hours, respectively.

STRAIN T McC

T McC was a culture given by Dr. A. T. MacConkey to Miss Robertson who gave it to me in 1916. The agar colonies depicted in the first photographs (plate 5, second row, center) were derived from a single colony in agar. The second photographs (second row, right) were later derived from the original culture without intermediate fishing, the batch of medium was a different one, but the conditions were the same. These photographs represent, in all probability, different biotypes, and the opaque form may have been derived from the other.

STRAIN T 220

This was received in meat culture, in 1918, from Miss Robertson who isolated it from a wound. With it Tulloch[7] made the agglutinating serum that enabled him to establish his type 3. It apparently existed in 2 forms in the meat culture as it was received, for transplants shortly from that culture have twice shown 2 forms (plate 5, third row). These have been worked with quite extensively and no other type has been noted in the cultures. It is not known which form gives rise to the other. Strong toxins that produced typical tetanus in mice were produced by both strains.

STRAIN VT

This was given me by Dr. K. F. Meyer, who secured it from a vaccine process in a fatal case of tetanus in a little boy who probably became infected through playing in a stable. When first studied, 2 very different types of colonies were noted that, when isolated, were photographed (plate 6, first and second rows). Strong toxins that produced typical tetanus in mice were formed by both strains.

THEORETICAL CONSIDERATIONS

Tetanus strains mutate readily in protein mediums in which they are able to multiply actively. This mutation is apparently much more frequent than in the case of nonproteolytic anaerobes. The phenomenon is in accordance with the hypothesis,[2] that proteolytic anaerobes are the products of a more far-reaching evolution than nonproteolytic ones. That is, the proteolytic anaerobes always have mutated more frequently on protein mediums than the others, and that is why they have attained their specialization.

Some strains of tetanus bacilli apparently mutate more readily than others.

The mutations of these organisms, frequent and striking as they are, never, so far as I have been able to observe, result in anything but the formation of typical tetanus bacilli. The derivation of forms differing from their ancestors in several important characters should be considered as possible only after the passing of a far longer period than that noted by certain workers with anaerobes (Grassberger and Schattenfroh;[8] Kolle, Ritz and Schlossberger[9]). A worker claiming to change a blackleg bacillus into a Welch bacillus would, in my opinion, in order to substantiate his claims, have to show pure cultures of several intermediate biotypes, for apparently mutation of several important characters does not occur, either at once, or in rapid succession. It is improbable that radical changes involving many characters of an anaerobe occur in short spaces of geologic time; otherwise we would not find universal common types all over the world.

As in the case of higher organisms, mutation may be either favorable or unfavorable to the existence of the organism. Probably numerous mutations affect the metabolism neither favorably nor unfavorably. The chances of detecting a favorable mutation are far greater than are those of detecting an unfavorable one. This does not correspond with the findings that Beijerinck[10] and others have made with aerobes;

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[8] Arch. f. Hyg., 1901, 48, p. 50; p. 1312; 1905, 53, p. 58.

[9] Med. Klin., 1918, 14, p. 281; München. med. Wchnschr., 1919, 66, p. 348.

[10] Folia mikrobiologia, 1912, 1.

Beijerinck believes that acquisition of characters by mutation has not been demonstrated for aerobic organisms. This is doubtless a matter of interpretation, but it is far easier to demonstrate the acquisition of a power by an anaerobe than the loss of one, when deep colony methods are used. The process of identifying mutations in the study of strain TO87 depended frequently on the detection of the mutation in the very colony in which it occurred—one among perhaps 100 or 500 others. This was easy because such colonies are bigger or fluffier than their neighbors, or they are asymmetrical. The chances of deliberately fishing a mixed colony in which a less capable mutant exists are remote—unless, indeed, there should appear an inhibitive or autolytic action like that noted for the colon bacillus by Bordet and Ciuca.[11] But in old cultures such less capable forms may exist and may be discovered by careful search. In the process of this investigation many minute colonies were fished before any were found that bred true. Colonies descended from spores and colonies near the wall of the tube may be smaller than their neighbors, but are usually of the same biotype.

No case of reversion has, in this brief study, been discovered. Beijerinck terms the reappearance of pigment forming prodigiosus colonies in nonpigment-producing strains "reversion." Pigment is a substance that may be analyzed and such reversions may thus be definitely proved. But when a mere matter of colony form is under consideration, it will not be wise to call the reappearance of an ancestral form in a pure mutant culture a reversion, unless we know something of the chemical nature of the processes involved.

According to Dr. Thomas Morgan (pers. com.), there are two kinds of possibilities that may determine reversion in bacteria, a recombination of two genes to produce a remote ancestral type being probably restricted to forms in which amphimixis occurs. The reversions which we should consider may depend on the tautomeric action of a gene that is always present and that may balance one way or another, or they may depend on an entirely new mutation that produces the same character as was possessed by an ancestral type. In the first case, the reversion will occur repeatedly in a certain percentage of cases; in the second case it will be exceedingly rare.

The study of mutating colonies will enable the systematist to deduce which metabolic characters are least constant by making special mediums containing substances that are to be investigated, and studying the colony formation in deep agar shakes made of such medium. This method is analogous to the well-known one of putting indicator in special sugar mediums for use with aerobes. If an indicator could be found that would not be reduced in the presence of anaerobic growth, it would be of great value in determining anaerobic mutations on carbohydrates. Such substances as furnish material on which the bacteria readily mutate, may be rigorously excluded from a standard medium on which the habits of various strains may be compared. An easy way is thus offered of avoiding expensive and time consuming tests of the behavior of certain organisms when such tests should never be used for systematic purposes on account of the mutation of the organisms under the conditions afforded by those tests.

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[11] Compt. rend. Soc. de biol., 1920, 83, p. 1293.

The significance of these mutations to the biochemist is great. His road is a difficult one to plan. He must make quantitative determinations and yet know that they may be impossible to confirm. Specific chemical action exists, surely, but it will be difficult to determine what is specific and what is not. The chemical behavior of anaerobes should be studied for several strains of the same group that differ in every known way.

Mutations are also of importance to the therapeutist. DeKruif [12] has recently found two types of the bacillus of rabbit septicemia living side by side, both virulent for rabbits, one far more so than the other. For organisms whose pathogenicity is dependent on their invasive power, such mutations are of great significance for purposes of immunization. Virulence depends very much on the ability of the organism to multiply in its environment. It has been customary of late for surgeons to depend, in certain types of tetanus cases, on antitoxin to help the body to fight off an infection that would otherwise remain somewhere near equilibrium, instead of operating to sterilize the seat of the infection. Theoretical considerations are radically against such procedure. Any day a new form of tetanus bacillus may arise that is four or five times as well able to multiply as were its ancestors, and this type may upset the equilibrium. Such a phenomenon may explain many cases of latent tetanus.

No effect of these observed colony mutations on toxin production has been noted, although it is reasonable to presume that by multiplying more extensively a strain will produce a stronger toxin. Filtered toxins prepared from some of the mutant strains had similar titers. All strains tested produced strong toxins in 3-day meat cultures except T 087 and its derivatives which behaved consistently with one another.

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[12] Jour. Am. Med. Assn., 1921, 76, p. 651.

Miss McRoberts of this laboratory is investigating the agglutination reactions of these strains and will report on them separately. One is forced to presume that these nutritional mutations are more frequent and superficial than such as would be discovered by immune reactions where a large protein fraction of the bacterium or a secreted toxin is the substance estimated.

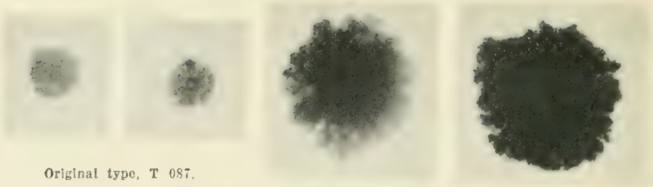
TECHNICAL DEDUCTIONS

In choosing anaerobic material for the study of mutations one may seek in the literature or in his own notes for evidences of irregularity in the behavior of certain types of organisms. The worker should first investigate whether the irregularities are due to contamination, for the literature is permeated with reports based on the behavior of contaminated cultures. If pure cultures have been found that behave peculiarly, a medium may be chosen that will emphasize the peculiarity. Deep colonies in this medium should be craefully observed, and, if the work is undertaken seriously, the colonies should be occasionally photographed. A species of organism should be chosen that does not form much gas or grow readily through agar mediums as though they were broth. Strains selected for particular observation should make symmetrical colonies that show little variation. A strain that normally is lenticular but occasionally sends out woolly processes, such a character being quite within the limits of variation for the biotype, is not well adapted for the study of mutation.

One should use the same batch of medium throughout a given experiment whenever possible (plate 5, lower series).

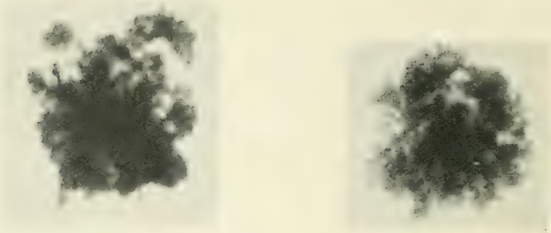
A type of photographic plate should be used that covers a wide range of lighting conditions and can always be procured. Detail, in the photography of colonies, is more important than contrast.

One should refuse to undertake to study mutations in a dusty room where there are draughts or people pass frequently—otherwise other types of anaerobes will probably make their appearance in the culture.

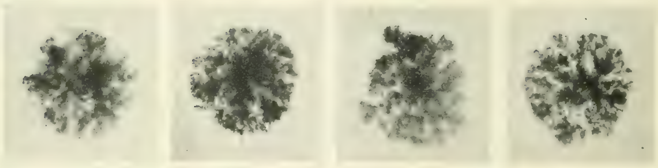


Original type, T 087.

TDW.

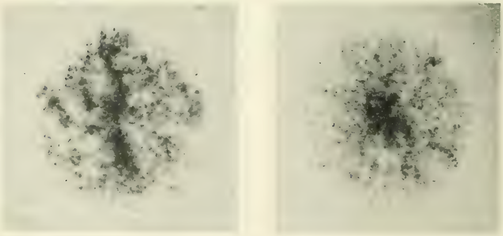


TOL.



TOM.

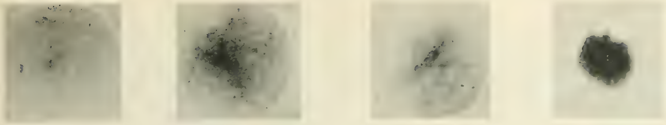
TRW.



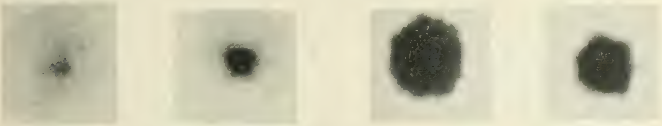
TLW.

Fig. 1.—The mutations of strain T 087. Five pairs of photographs show what are probably 4 types of mutants derived from the minute type in the upper left hand corner. They arose during two weeks' study of the behavior of this strain on deep liver peptone agar. All were grown simultaneously for 24 hours on the same batch of medium under identical conditions; colonies few; × 50.

Unless otherwise indicated this and the following illustrations represent colonies that were incubated for 24 hours at 37 C. in deep liver peptone agar and were enlarged 50 diameters.



Strain TT, probably identical with strain T USA; colonies not numerous, 1919.



Strain
T USA W.

Colonies not numerous, 1919.

Strain
T USA S.

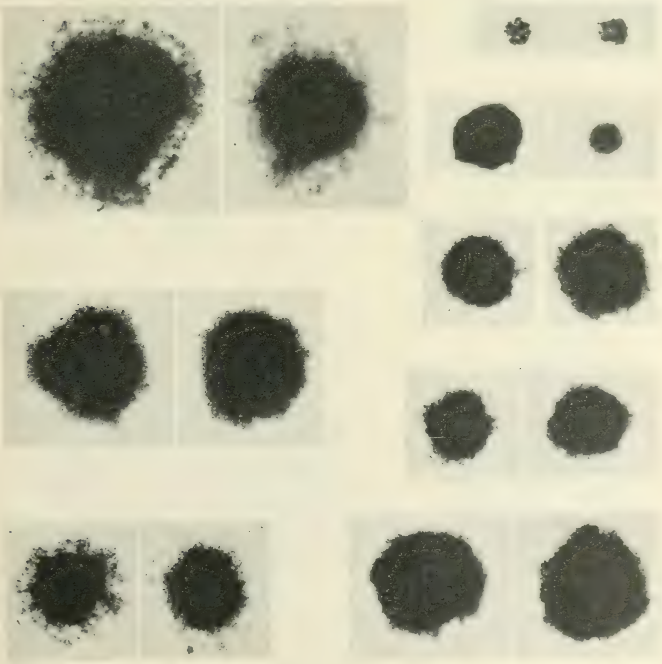
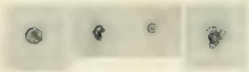
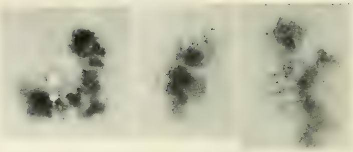


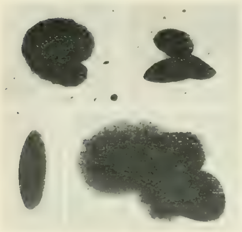
Fig. 2.—Mutations of strain T USA. In October, 1920, a meat culture of T USA (W) showed the fluffy type only. In March, 1921, the same meat medium tube that had long stood at room temperature showed a number of biotypes. The original form was apparently lost; some new types were larger than the original; all were denser and two smaller. The conditions of growth for the members of each of the three series were identical. The lower series were all taken on a Seed No. 23 plate; colonies few; × 15. The upper series were taken on softer plates at 50 diameters and were reduced to 15 diameters.



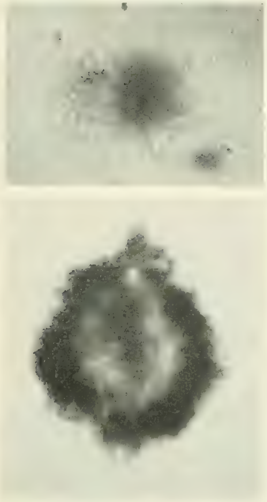
July 3, 1919; colonies moderately abundant; × 50

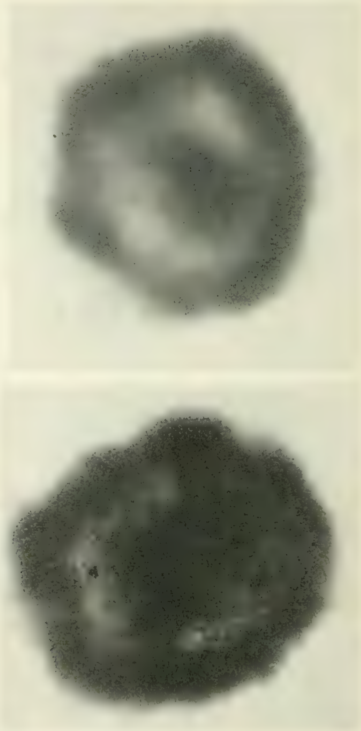


Oct. 2, 1919; colonies few.



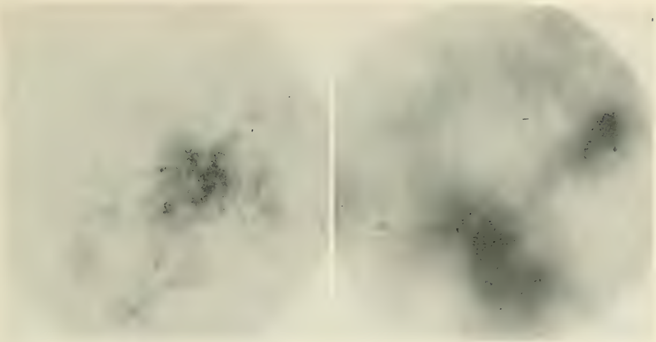
The above type 5 days at 37 C.; colonies few; × 15.



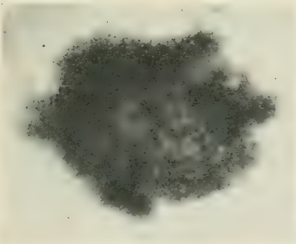


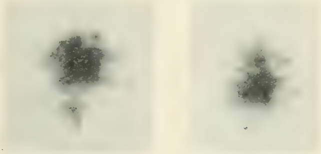
March, 1921, colonies not numerous.

Fig. 3.—Changes in the behavior of strain TG. The origin of the woolly form from that of Oct. 2, 1919, was observed. This is a type with great range in size of colony.



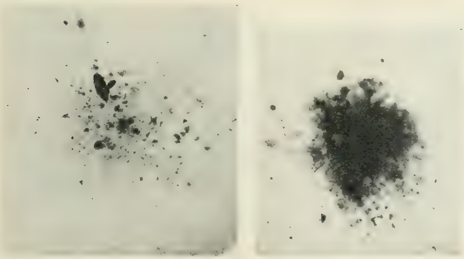
Oct. 28, 1919. Colonies few. December, 1919.

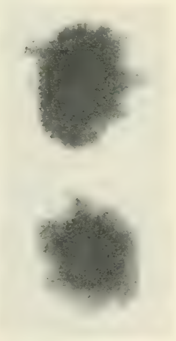




Oct. 28, 1919; colonies few; × 15.

Oct. 22; colonies few; × 15.





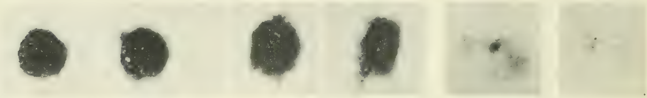
Strain TVP F. Strain TVP D.

Colonies few; × 15.

Fig. 4.—The behavior of strain TVP. The upper 5 photographs show the inconsistent behavior of the strain. The lower ones, taken under identical conditions, show 2 types isolated from the original material in April, 1921.

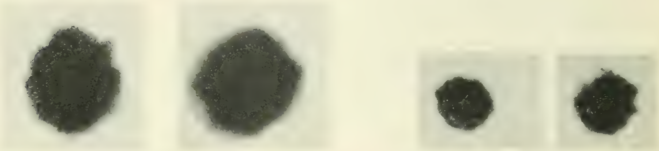
Colonies few; strain TL, December, 1919.

Colonies not numerous. Sole colony. Strain TLB, April, 1921.



Strain TLC, April, 1921; colonies few.

July 7, 1919. Sept. 30, 1919. Strain TMcC.



Strain T 220 W. Strain T 220 S.

Colonies not numerous.

Pairs of types noted at various times.

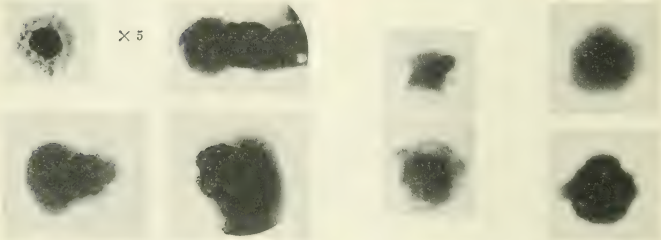
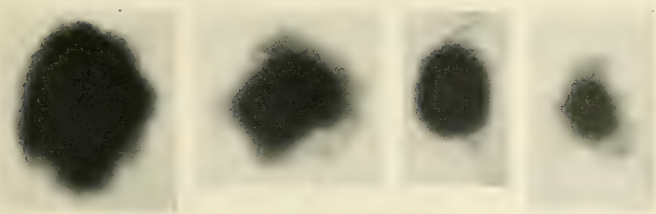
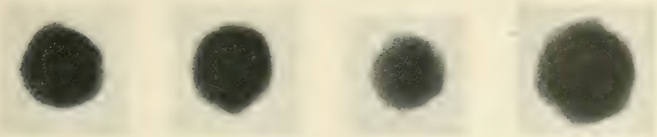


Fig. 5. Simulated mutation strain T 220 S. This strain forms in 5 days colonies like the first (× 5) of this series. Cultures from the center gave dense woolly colonies (on the right) on stiff agar; cultures from the edge gave compound lenticular ones (the 3 flat colonies on the left) on soft agar. Subsequent transplants on the same batch of medium showed that the strains were identical. The two middle forms are from an intermediate medium. Colonies few; 48 hours old; × 15.



Strain VTW; colonies few. Colonies moderately abundant.



Strain VTS; colonies few.

Strain VT gave the two types when first observed in meat culture.

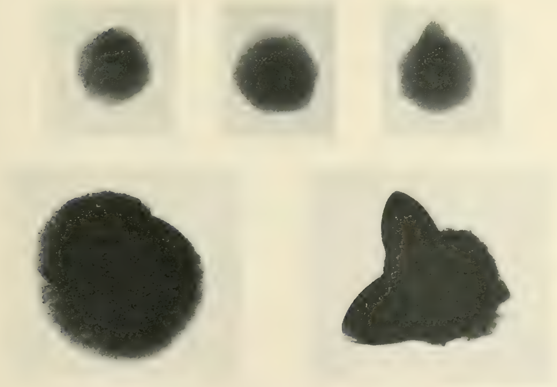


Fig. 6.—Strain TA: the upper colonies were taken in June, 1919, the lower ones in April, 1921, under approximately identical conditions; colonies few; × 50. It is obviously impossible to determine from these data whether mutation occurred or not.

The Study of Colony Formation in Deep Agar

STUDIES ON PATHOGENIC ANAEROBES VI

HILDA HEMPL HELLER

Reprinted from
The Journal of Infectious Diseases, Vol. 30, No. 1, Jan., 1922, pp. 1-17

THE STUDY OF COLONY FORMATION IN DEEP AGAR *

STUDIES ON PATHOGENIC ANAEROBES VI

HILDA HEMPL HELLER

From the George Williams Hooper Foundatiin for Medical Research, University of California Medical School, San Francisco

Under uniform conditions living creatures of the same species vary in shape only within comparatively narrow limits. Morphology is, in the case of the majority of living things, the character that is the most easily observable, and for this reason it has been used as a basis for the classification of all forms of life. Among the bacteria such use of morphologic criteria for systematic purposes is frequently avoided because the form of these organisms is exceedingly simple and because the bacteria are highly specialized, not as to shape but as to the enzymes with which they split the substances surrounding them. Higher plants are classified not according to the shape of their stems and leaves, but according to that of their reproductive organs. For purposes of bacterial classification, we may well let chemical characters take the place of those morphologic ones that the bacteria do not possess. A chemical classification will then serve to build a skeleton structure which will group and divide the bacteria into genera [1] that are comparable with those of the higher plants. The trend followed in dividing the bacteria has usually been to employ morphologic characters for the making of major divisions and chemical ones for the finer differentiations. For many reasons pointed out in a former paper [2] morphologic criteria fail to furnish a means for logically subdividing the major group of anaerobic rods. Chief among these are the facts that most morphologic characters appear in various groups, that there is a general overlapping of such characters and that there is a lack of correspondence between chemical and morphologic differ-

Received for publication, July 5, 1921.

* The present paper is intended as an introduction to others in which the colony formation of anaerobic strains will be discussed.

[1] Heller, H. H.: J. Bacteriol., 1922, 7 (Jan.).

[2] Ibid., 1921, 6, p. 521.

ences. It is the purpose now to indicate the exploitation of a so-called morphologic character for purposes of finer differentiation.

Anaerobic bacilli form within a solid nutrient agar mass colonies of very different shapes, but many types are constant in their behavior if the conditions surrounding the organisms are uniform. This constancy is such that if a strain of anaerobic bacilli be inoculated, on various occasions, from a culture of the same age grown on a similar medium, always onto the same variety of deep agar, and if it be incubated for the same period at an identical temperature, it will produce colonies that are enough alike to furnish the systematist with an excellent means for the comparison and identification of cultures. This constancy is such that the slight variations in conditions that occur when technic is faithfully imitated by conscientious workers in different laboratories are not sufficient seriously to impair the use of deep colonies for systematic purposes in certain of the anaerobic groups when simple mediums are employed. The colonies are similar enough to warrant their comparison to the leaves and stems of higher plants. The same overlapping of characters of vegetative portions appears among higher plants as among bacterial colonies. Thus some senecios look more like cacti than they do like other senecios; the foliage of a hakea resembles that of some accacias more than that of other hakeas; remarkable resemblances of foliage occur among members of the genera Juniperus, Cupressus and Chamycyparis. But sort out the senecios and the hakeas on the basis of the morphology of their reproductive organs, then their leaves and stems differentiate their species from each other; sort out the bifermentans from the Welch bacilli and the vibrion septique from the sporogenes on the basis of their chemical reactions, and the morphology of their colonies will serve to group strains that have thus been sorted into genera into smaller groups which may or may not be considered as species, depending on the constancy of colony formation in those genera. The only great difference in such a comparison between the higher plants and the bacteria is that the latter, immersed in gels containing liquids of widely different composition, are far more subject to such variation as is determined by external conditions than are the former.

Surface colonies of aerobic organisms have been used for systematic purposes and for the study of mutations for many years. Such colonies are frequently characteristic in shape, but colony characters other than form, such as pigment formation and opacity, have been frequently used for these types of investigation. The aerobe surface colony is much more characteristic and uniform in contour than the anaerobe surface colony. The former is automatically limited in size because grown in the dry air of the incubator, while the latter is subject to such different moisture and gas conditions that though specifically fairly characteristic, it is not very uniform in behavior. Anaerobic surface colonies have been described in most detail by McIntosh,[3] Henry,[4] Adamson[5] and Zeissler.[6] The latter has used colonies on glucose blood agar as a basis for the subdivision of the vibrion septique group;[7] his method has the advantage of showing hemolysis. In the depths of an agar medium gas and moisture conditions are so controlled that their disturbing effects are eliminated, and colonies growing therein are more uniform in behavior, and their study permits much more accurate determination than does that of surface growths.[8] Deep colonies have been figured most extensively by von Hibler[9] and by Weinberg and Seguin.[10]

Illustration of the way in which certain of the anaerobic genera may be subdivided by colony determination will be given in subsequent papers. A collection of colony photographs of organisms that do not belong in the groups to be later discussed is included in the present paper (plates 1-5).

The shape of a bacterial colony is not to be thought of as a matter of chance nor, on the other hand, is it in any degree so dependent on the inherent germ plasm as are the shape and histologic arrangement of the tissues of the higher plants. It is dependent, first, on the moisture content of the medium, secondly, on the texture of the colloid mass as determined by the rapidity of cooling, thirdly on the ability of the organism to multiply in the medium, and fourthly on the motility of the organism in the medium. Intrinsically characteristic generic or specific colony form, if it exists, is subordinate to these four factors. All the chemical phenomena that are observable in bacterial growth in liquid medium must be thought of as contributing to shape the colony in a solid medium. Pabulum—split proteins, sugars and vitamins;

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[3] Med. Research Committee, Special Report 12, 1917.

[4] J. Path. & Bacteriol., 1917, 21, p. 344.

[5] Ibid., 1919, 22, p. 345.

[6] Ztschr. f. Infektionskr. d. Haustiere, 1920, 21, p. 1.

[7] Heller, H. H.: J. Infect. Dis., 1921, 27, p. 385. Reprints, Hooper Foundation, 1921, 6.

[8] Heller, H. H.: J. Bacteriol., 1921, 6, p. 521.

[9] Ueber die pathog. Anaeroben, 1908.

[10] La gangrène gazeuse, 1918.

lag; initial reaction, buffers, acid inhibition, neutralization by alkaline end-products. Temperature, acid, and autointoxication have their effect on motility as well as on growth.

Therefore the acquisition by an individual bacillus of the ability to split and assimilate a nutritive element present in a substratum will make itself known by an increase in the size of the colony formed by the mutating bacillus. Likewise the loss of a power involved in the assimilation of nutriment from a medium will cause the formation of smaller colonies in that medium. Other mutations involving the formation of autointoxication products, the motility, or even the rate of disintegration of bacillary bodies, will also affect the size or transparency of the colonies.

Colony shape may then be used in the case of anaerobic bacilli as a technical means of spotting mutations. Mutating forms may then be isolated, studied on the medium in which they appeared, and suitable experiments may then be arranged to demonstrate, if possible, what was the metabolic cause of the change in colony form.

As emphasized before,[2] the mutations of anaerobes, though little studied, cannot be essentially different from those of other living things. Careful work makes it seem highly improbable that such mutations ever involve many characteristics at once. The anaerobic bacilli are relatively stable types. A Welch bacillus cannot be changed into an amylobacter, nor a blackleg bacillus into a Welch bacillus or sporogenes bacillus or aerobic bacillus. On the other hand, to claim that mutations do not occur among these organisms would be to state that they lack one of the best recognized attributes of living matter. So far as we know, they have originated by descent and not by spontaneous generation—if not by mutation, then how? Apparently mutation, such as may be recognized by a change in colony form, occurs far more frequently in some groups of anaerobic organisms than in others. In such groups colony form is not available for systematic purposes.

A colony differing from its neighbors, when fished onto a new series of agar tubes, may or may not give new colonies like itself. For the observation of such colonies a control series of the normal type present should always be inoculated, and the appearance of the colonies should be noted at several different time intervals. Occasionally two colonies will be found in a culture derived from a single colony, whose descendants pass through the same morphologic history.

but do so at consistently different speeds. There is, then, a difference between these strains, and the new form may be accepted as a mutant of the other. On the other hand, one may often fish two colonies of different appearance from a tube containing a considerable number of colonies, and their offspring may be all alike or show similar variations that are clearly not mutations. One more set of dilution tubes will usually be sufficient to settle the question whether a mixture of two types is present or whether the differences noted lie within the limits of variation for the species. Some anaerobic strains occasionally show in thickly seeded tubes one or a few colonies several times larger than the others. These are usually constellations of small colonies derived from minute chunks of infected inoculum. When planted on a new series of tubes, the same forms may appear in about the same proportion as before, and repeated selection from such colonies does not increase their number. These large colonies do not breed true and are thus not mutations.

TECHNICAL CONSIDERATIONS INVOLVED IN THE STUDY OF ANAEROBIC COLONIES

Inoculation of material for photographic representation of the colonies hereafter pictured was as follows: Liquid from meat cultures was used for inoculum. In the case of rapidly growing anaerobes one or two day cultures only were employed. In the case of proteolytic organisms a somewhat older culture was occasionally used, but old run out cultures were avoided. Tubes of agar were melted in the water bath and were then tapped and shaken sharply and replaced in the water bath for a few minutes and then shaken again. This method removes the air without soaking the plugs with steam. The tubes were then cooled to 42 C., and the first was inoculated with a small loopful of culture and shaken; the second was usually inoculated from the first with the amount of agar that would fill the capillary of a large bore Pasteur pipet; the pipet was then well flamed, and the third or third and fourth tubes were inoculated with somewhere between 0.25 and 0.75 c c of material from the second tube. Colonies were well distributed by rolling and tipping. The tubes were allowed to cool in air at room temperature.

Twenty-four hours was chosen as the standard time for incubation unless there was a reason for choosing another period. Thus blackleg colonies are invisible at 24 hours, as are those of some strains of tetanus. For some organisms a comparatively early stage is characteristic and for others a later stage is more so.

Preparation of colonies for photographing is carried out as follows: The tube containing colonies to be photographed is held with the mouth slanting toward an open Petri dish, and an open large bore Pasteur pipet bent at a right angle is inserted along one side to the bottom. This pipet is then removed and the agar bdown out of it, and it is then reinserted and through it the column of agar in the culture tube is blown out into the Petri dish. A safety razor blade may be used to section the medium, usually at right angles, and slices about 2 mm. thick are placed on a slide with a drop of water and covered with a small coverslip. These are examined directly on the ground glass of the camera.

Photographic Technic: The microscope and camera are arranged on the plan designed by Dr. L. B. Wilson,[11] the lens of the camera being removed. The source of illumination for most of the pictures was a Spencer light placed under the stage of the instrument. Mirror and condenser were removed. A ground glass disk such as accompanies Leitz microscopes was placed on the stage under the slide. For later work a nitrogen light[12] and a mirror without condenser have been employed. The ocular used in all cases has been a Zeiss projection ocular No. 4, and two objectives have been employed, Zeiss No. A_2 arranged to give on the ground glass of the camera a magnification of 15 diameters, and a Leitz No. 3 which gives an enlargement of 50 diameters. For work in which a wide range of lighting conditions must be recorded, such as one finds in general colony work, Seed's "Non-halation-L-Ortho" plate has been found superior, and Seed's "26" plate is also excellent. These two are fairly rapid and give, apparently, a maximum of detail in contour and in the minute fuzzy halos surrounding many colonies. For representation of the interior structure of smooth-contoured colonies, Dr. K. F. Meyer tells me that he finds "Process" plates excellent. Their range is not sufficient for general work. Seed's "23" plate also gives too much contrast for most purposes. Orthonon plates were found to be too thin. All the exposed plates were developed in a tank according to the directions accompanying them. It is well to choose a type of emulsion that may always be had and to use that type constantly for comparative work. Regular glossy Velox paper was used for the prints.

All conditions regarding age of colonies and age of inoculum, medium, incubation temperature and photography, are made as uniform as possible and are carefully recorded.

Contamination.—For ordinary laboratory purposes it is sufficient for the investigator of anaerobes to inoculate his cultures in a small quiet room where no other people are moving about. For a worker with considerable practice and skill contaminations occur rarely under such conditions, at least in an atmosphere that contains little dust. It is hopeless to try to do accurate work in a busy room.

Crowding.—The number of colonies present in a tube has a striking effect not only on the size but also on the shape of the colonies in the tube. Many types of colonies originate as lenticular masses and then send out protuberances or downy strands. If colonies with such a habit are abundant, they pass through the various morphologic phases in a shorter period of time than if they are few, and different colonies in the tube pass through their development at different speeds, so that from a single tube with many colonies the entire history of colony formation may be traced. This point is illustrated by the series of photographs showing the process of colony formation of a *Rivoltillus*[1] (vibrion septique) strain (Plate 6). Where colonies are numerous they are, of course, smaller than where they are few. For these two reasons therefore, types should always be chosen from tubes with so few colonies that they do not interfere with each other. A striking case of difference not only in form of the colony but in its texture is shown by the series of photographs of colonies of an unidentified anaerobic organism (Plate 7). To designate the number of colonies present in a tube, I have usually employed the following rough scale:

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[11] Spencer photomicrographic outfit 4336B.

[12] Bausch and Lomb adjustable microscope lamp No. 1784.

Sole colony	1
Colonies few	1— 20
Colonies not numerous	20— 60
Colonies moderately abundant Photographed for history of development	60—400
Colonies, abundant Rarely photographed	over 400

Permeating Growth.—The more rapidly proliferating species of anaerobes, motile and nonmotile, frequently leave their colonies and multiply in the agar gel as though it were a broth.[8] When this happens the colonies abruptly stop growing. Tubes containing this permeating growth may be readily identified by holding them to the light with a control. When there is more than a suspicion of this phenomenon the colonies should not be used for systematic measurements unless the permeation of the medium is a constant character of the strain. Plate 8 shows the effect of permeating growth on the size of the colonies. Colonies in agar cooled rapidly in water or ice water are more sharply delimited than those in agar that is cooled at room temperature. Uniform procedure should be adopted with regard to cooling the agar, or if the procedure be varied to diminish permeating growth note should be made of the fact.

Uniform Conditions.—An instance may be given of the difficulties encountered by the student desirous of identifying an anaerobic strain. No worker has time to duplicate the mediums and technic of von Hibler,[9] who gives excellent photographs of colonies in his book. Plate 2 of his book may be taken as an example. Among the mediums used for the growth of the colonies represented on this page are glucose cat-meat agar, spleen-broth agar, sodium formate spleen-broth agar, rabbit-meat agar, guinea-pig-meat agar, and for illustrations on other plates fish-meat agar and various other queer concoctions have been employed. Far better be it to give the conditions of growth as von Hibler does than not to give them at all. One appreciates his effort to study the organisms under different conditions, but he made his work hopeless to follow. He gives us a single page with 4 pictures of the morphology of 3 species of bacteria in the animal body. Then come 12 photographs of deep colonies and two of surface colonies of 8 species of organisms. These are photographed at 10 different ages ranging from 10 hours to 3 days, the organisms have grown on 9 different mediums from 4 or more different kinds of animals (it is to be hoped that he never confused his mediums) represented in 7 different enlargements. The anaerobist working on bibliography is surely a creature to be pitied, and is not to be blamed for failing to follow the work of others conscientiously and thoroughly. Von Hibler is also to be criticized for terming colonies formed on one kind of medium "typical" and on another kind "atypical."

Photographs of colonies should always be accompanied by the name of the strain that gave rise to them. The beautiful lithographs of oedematiens colonies shown by Weinberg and Seguin [10] represent colonies very different in aspect, but there is no indication which forms are characteristic for the various strains that another worker might consider to belong to distinct species.

One photograph is usually insufficient to give a fair idea of the possible variations of a strain. Several colonies should be depicted, the number depending on the limits of variation in the strain.

The medium chosen should be one on which as many species as possible will grow, but a universally suitable one is probably not to be hoped for. Liver agar has been found eminently satisfactory for most types of anaerobes but affords too fertile a substratum for some. It was excellent for producing a suitable colony formation with blackleg, oedematiens, tetanus and botulinus organisms. Vibrion septique strains grew too rapidly in it so that at 24 hours colonies descended from spores were smaller than those descended from vegetative rods; at 48 hours, they were uniform in size but permeating growth was so frequent that many tubes had to be discarded, and the growth was so luxuriant that the colonies of different strains could not be so nicely grouped as in the case of other genera. A search should be made in such cases for a less nutrient substratum or for a firmer medium. But there are indications that liver agar is so complex a medium that it furnishes too much chance for mutations to affect the shape of the colonies, and simpler mediums may have to be adopted. Agar more than a month old should not be used for colony photographs as it is too dry. For most types of anaerobes sufficiently uniform colonies may be secured on medium from 1 to 30 days old. Blackleg strains will not grow after the medium is from 6 to 10 days old. Agar that is too fresh or that contains too much moisture will also give misleading results (Plate 7). Such agar may be incubated to dry it out before using.

Liver Agar.—This is made up of beef liver 1 part, distilled water 4 parts. Infuse over night in the refrigerator, bring to a boil, strain through cheesecloth, add 1½% Difco peptone, 0.5% salt and 2% agar. Melt the agar, titrate and adjust to P_H 7.2, cool to 60 degrees, add white of egg and serum; autoclave for one hour, titrate again and adjust to P_H 7.2; filter through cotton, tube, and sterilize in the autoclave.

CONCLUSIONS

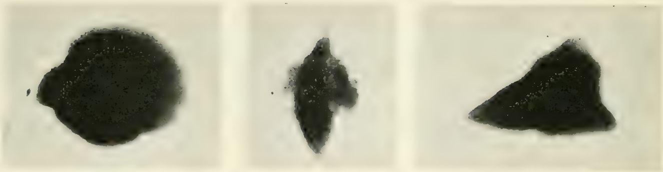
Under constant conditions members of a pure strain (biotype) of anaerobic bacteria make deep colonies that differ from each other scarcely more than do the vegetative portions of individuals of a species of higher plants.

It is found that a collection of anaerobic bacilli that correspond in all ordinary cultural characteristics may be broken up into groups of strains on the basis of colony formation in deep agar. These groups are, in some genera, when the medium employed is simple, to be termed "species," in others not. The observation of colonies may thus be of great value to the systematist.

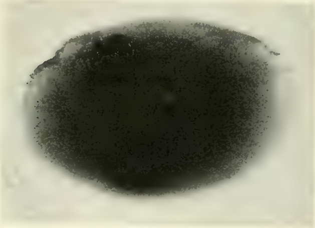
The shape of a colony in an agar medium depends primarily on the reproductive power of the organism and its motility in that medium. The reproductive power depends on its enzymes and metabolic activities. Therefore, any mutation involving the metabolic activities of the organism on a given medium may demonstrate its occurrence by causing a change in the colony morphology of the mutating organisms in the strain. Colony formation in deep agar is, then, a phenomenon whose study will be most closely observed by the student of mutation.

The colony shape must thus be thought of as a chemical character as well as a morphologic one, and the possibilities of its exploitation on specially prepared mediums to test the nature of mutations are infinite.

Technical considerations and various pitfalls are discussed: preparation of colonies and their photography, contamination, crowding, permeating growth, uniform conditions and choice of medium.



Bacillus 12 of McIntosh, type strain. Colonies not numerous; × 50.



An unidentified mildly proteolytic organism isolated from stools of infants by Miss J. Easton. Colonies few; × 50.

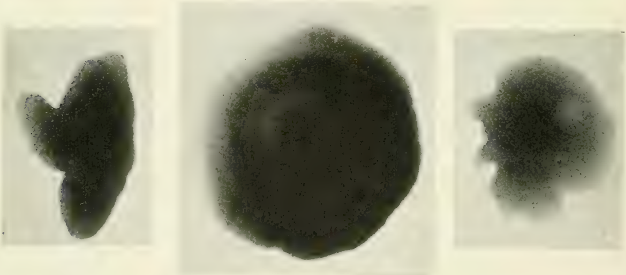
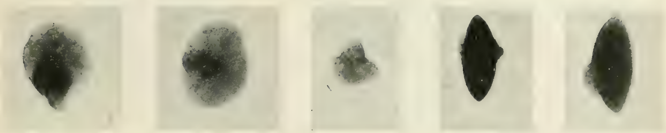
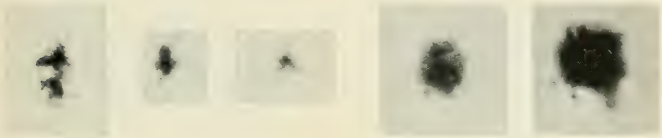


Fig. 1.—Lenticular and modified lenticular types. Except where otherwise noted, the colonies photographed in this series were grown on beef liver peptone agar for 24 hours at 37 degrees. The nomenclature employed is explained in another paper.[1]

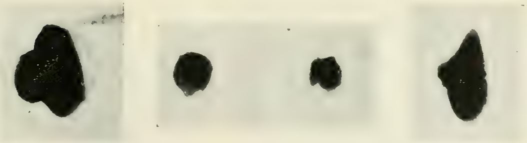


Putrificus sp.; strain IOR from septic wound. This corresponds more nearly to Bienstock's description of B. putrificus than any other strain in the collection. Colonies moderately abundant; × 50.

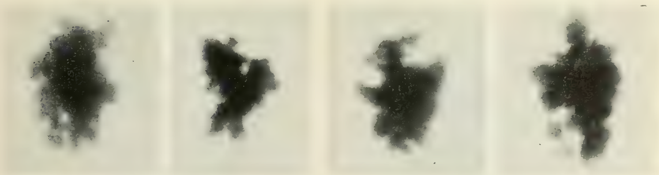


Many colonies present, 18 hours. Few colonies present, 24 hours.

Weinbergillus histolyticus, type strain, HW; × 50.

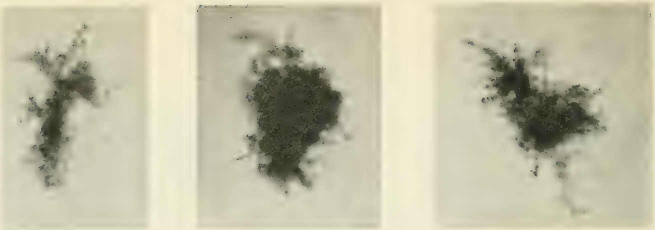


Seguinillus aerofoetidus, type strain, AW. Colonies few; × 15.

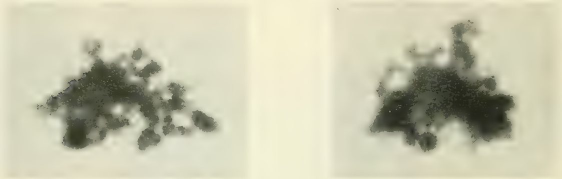


Vallorillus fallax, type strain, FW. Colonies few, 4 days old. Note diffuseness of outline due to permeating growth; × 50.

Fig. 2.—Colony forms.

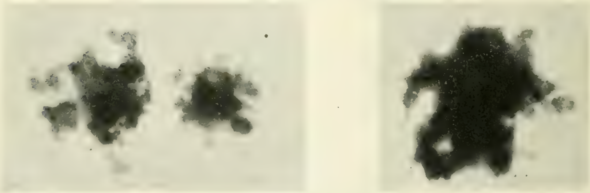


Martellillus proteolyticus; type strain, F, from septic wound, man. Colonies few; × 50.

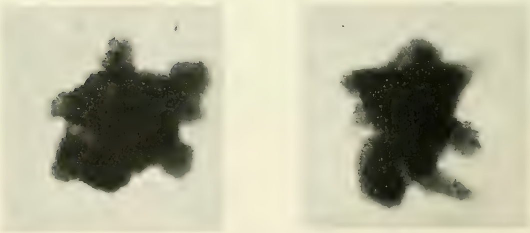


Martellillus sp. Strain JC, from Bradsot material. Conditions as above.

THE BIFERMENTANS TYPE

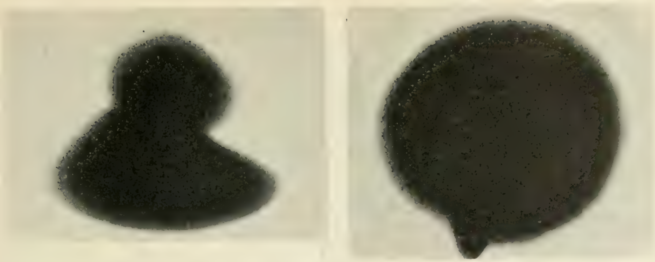


Eighteen hours, colonies few.



Twenty-four hours, colonies moderately abundant.
Robertsonillus primus. Type strain, L, from septic wound, man; × 50.

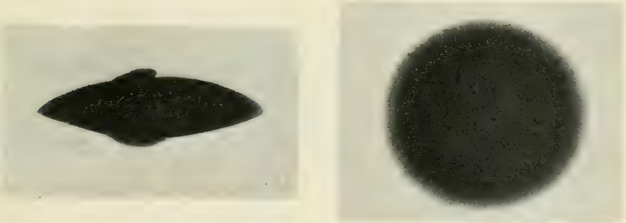
Fig. 3.—Colony forms.



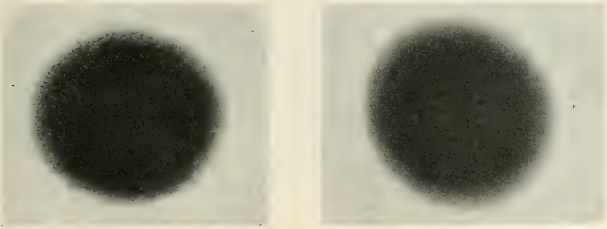
Strain WM; few colonies.

Strain WAT; few colonies.

Welchillus (B. Welchii) colonies; × 50. Note dulness of outline due to permeating growth.

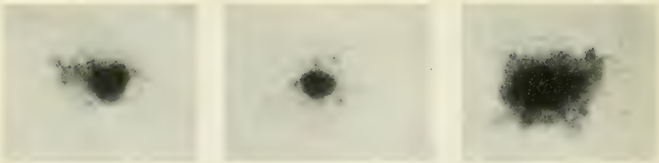


Strain P 11; isolation of Robertson; few colonies.

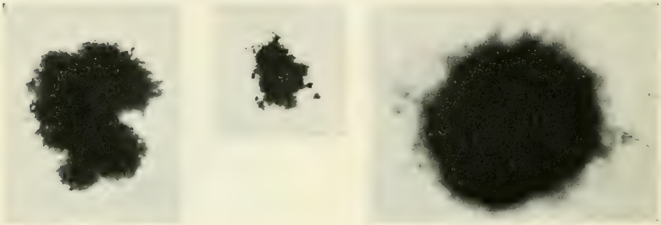


Strain P 7; isolation of Robertson; few colonies. Henrillus (B. tertius) colonies; × 50.

Fig. 4.—Nonmotile organisms.



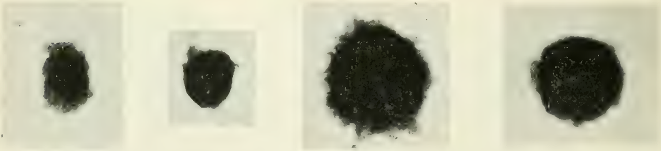
Eighteen hours old, colonies not numerous; × 50.



Two days old, not numerous; × 15. Note difference in size of colonies.

Veal infusion agar; eighteen hours; × 50.

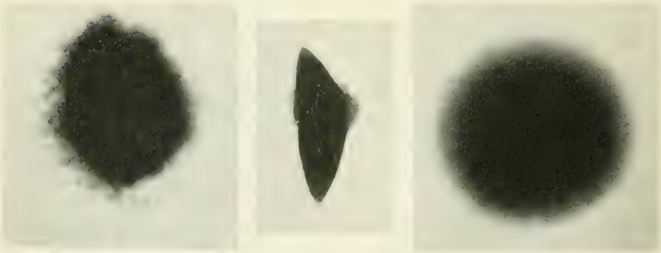
Metchnikovillus sporogenes; Strain MR; isolation of Robertson.



Eighteen hours; × 50.

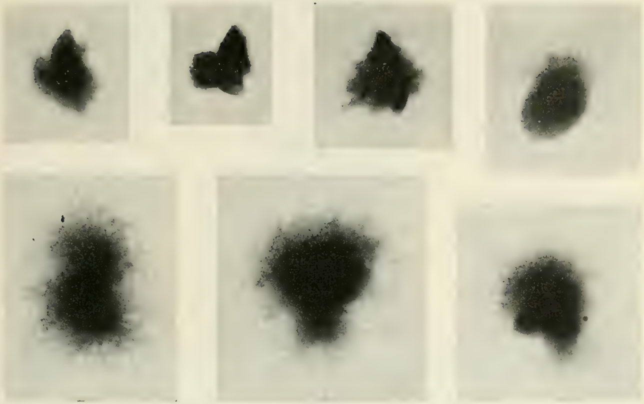
Eighteen hours, veal infusion agar.

Metchnikovillus parasporogenes McIntosh; strain R 60.

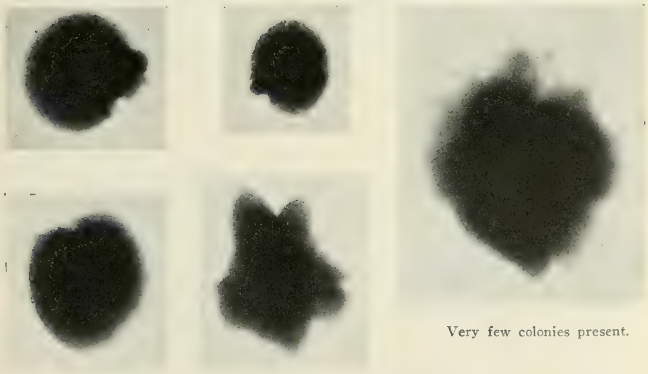


A culture of "Malignant oedema bacillus" from Germany resolved itself into two species, a typical sporogenes strain (at the left) and a nonmotile less strongly proteolytic organism (at the right).

Fig. 5.—Highly motile organisms.

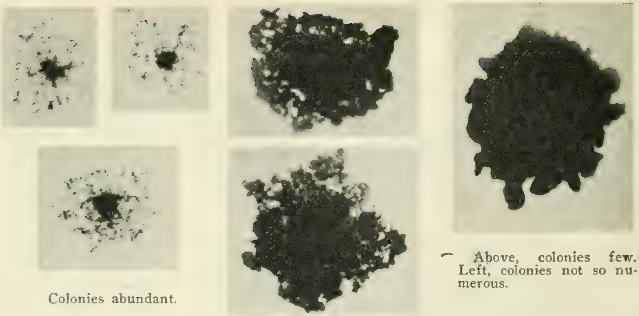


Colonies moderately abundant.



Very few colonies present.

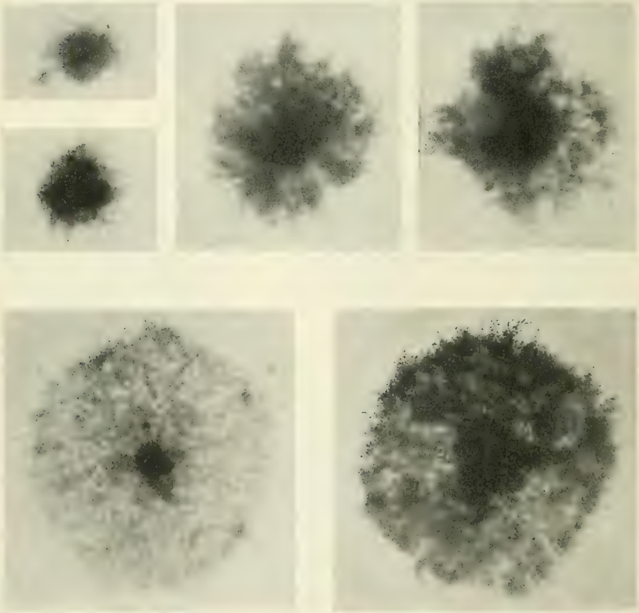
Fig. 6.—The development of a vibrion septique colony. Vibrion septique strain VS that retains its lenticular form unusually long (24 hours); × 50. The influence of crowding is well shown. The second group of colonies developed in a tube inoculated from the first. A greater dilution of inoculum was the only difference in the conditions in the two tubes.



Colonies abundant.

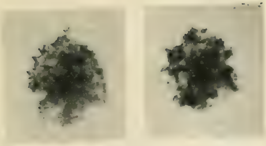
Above, colonies few. Left, colonies not so numerous.

An extreme example of the influence of crowding. An unidentified organism isolated by Miss J. Easton. Three successive dilutions of a pure culture; × 50.

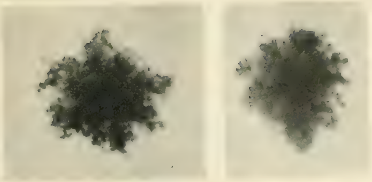


An instance of the influence of moisture in the agar on colony formation. Tetanus strain T 087, grown under otherwise identical conditions in "2%" agar that was (above left) old and dry (above right), fresh, and (below) unusually soft.

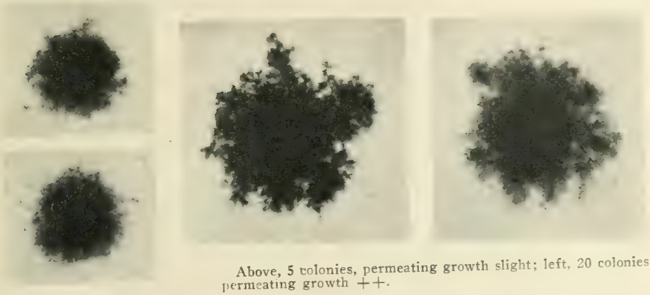
Fig. 7.—Influence of crowding and of moisture.



Strain HV; 7 colonies; permeating growth +.

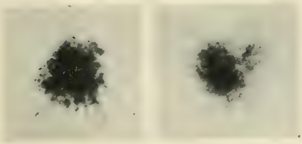


Eight colonies; permeating growth —.

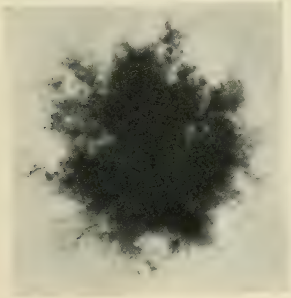


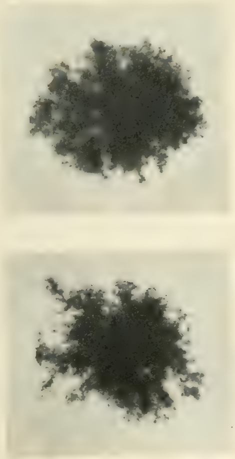
Above, 5 colonies, permeating growth slight; left, 20 colonies, permeating growth ++.

Strain AS



Strain BN; 3 colonies; permeating growth slight.





Sole colony, permeating growth —. Twenty colonies, permeating growth —.

Fig. 8.—The influence of permeating growth on the size of the colony. Vibrion septique colonies 48 hours old; × 50.

Reprinted from THE JOURNAL OF BACTERIOLOGY
Vol. VII, No. 1, January, 1922

CERTAIN GENERA OF THE CLOSTRIDIACEAE

STUDIES IN PATHOGENIC ANAEROBES. V

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Received for publication March 1, 1921

In a previous paper a classification was suggested for the group of anaerobic rods which include, roughly, the anaerobic members of the genus *Bacillus* of former workers. A family was proposed for these organisms, with the name *Clostridiaceae*,[1] and it was divided into two subfamilies, the *Putrificoideae*[2] or proteolytic anaerobes, and the *Clostridioideae*[2] or non-proteolytic anaerobes. Certain genera for these groups are proposed in the present communication. These in some cases unite various described species, while in other cases the genera themselves correspond to the former idea of species.

A key to the genera is also given, which is based on the action of the anaerobes on meat medium and on their general cultural behavior and morphology. Possibly the main lines for tribal organization will to some extent follow this key, but it pretends to be no more than an artificial arrangement. It is in no way complete and cannot be implicitly relied upon for purposes of classification. It is meant more as an index to the forms whose descriptions are sufficiently clear to warrant assigning them definite positions. The key will serve, for a time, as a nest of pigeonholes in which to place new species until material is sufficient for a complete reorganization, but workers should not try to place all newly discovered organisms in these genera.

The conservative worker, familiar with aerobic pathogens, who enters the anaerobic field, is all too prone to wish to "identify" his anaerobic strains. A natural feeling exists among systematists, that the description of a new-found type as a new species is to be avoided unless the describer is very sure that his type differs from all others. There is a fear that a description carefully made will some day be discarded by a future taxonomist who finds it identical with a former description. This logical attitude has bred and fostered a wholly unscientific mania for identification of all new strains with organisms already described by others. In the case of parasites or in well-known groups this procedure will only occasionally lead the worker astray. In the anaerobic field it will do so frequently. The scientific attitude relative to the taxonomic affinities of anaerobic bacilli is to state the group or generic relationships of the organisms and then to describe them minutely. The routine bacteriologist should be content to assign an organism to its proper genus.

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[1] For definition see Jour. Bact., **6**, 536.

[2] For definition see Jour. Bact., **6**, 550.

Colony formation in deep agar is included in the descriptions of genera, but a restriction of the genera to such types of colony form as are mentioned would be unwise. The definition of the Gram staining reactions should not be regarded as of great value in these descriptions, as the staining reactions of the organisms and the technique of workers are too variable. Sugar fermentations may be relied upon in the case of actively growing species only, and are valid only for media on which the behavior of a strain is constant. The constancy of the fermentations here quoted has not been verified by myself. Pathogenicity is not to be taken as a criterion for admission to a genus.

It is to be feared that bacteriological systematists who are unacquainted with the anaerobic group will object to the creation of so many genera as are here proposed. The Committee on Characterization and Classification of Bacterial Types (1920) propose but 38 genera for the orders *Actinomycetales* and *Eubacteriales*. The influence which the medical history of the science of bacteriology has had on this classification is marked. Two-thirds of the 38 genera recognized contain parasites. If we regard the anaerobic bacteria as wild plants growing in soil, as we have every reason for doing because of their many species, we must classify them as do the botanists and not as do the bacteriologists. The family of *Orchideae* has 334 genera, the *Gramineae* 298, the *Rubiaceae* 377, the *Leguminoseae* 399, and the *Compositae* 766 (Bentham and Hooker). The following classification imitates the taxonomic arrangement of botanical classifications in preference to bacteriological. It must be borne in mind that the date 1920 in bacteriologic systematics corresponds roughly to the date 1760 in botanical systematics.

Relative to the evolutionary history of the anaerobes we know nothing, but conjectures are not entirely out of place. Proteins and carbohydrates have existed together and have been destroyed by bacterial action since a remote geologic epoch. We cannot tell whether the organic catalyzers of one type of substance antedated those of the other or whether they are closely related. Botanists are not agreed as to what form of life first tenanted the globe. When looking for ancestral forms it is natural to seek in a group of organisms of great catalytic activity like the *Clostridiaceae*, for types which may synthesize simple substances, and if such are discovered it is not out of place to regard them as more closely related to the hypothetical ancestors of the group than those that do not synthesize inorganic substances. Such reasoning is of course entirely dependent on the homogeneity of the group under discussion. We have no means of proving the homogeneity of the *Clostridiaceae*. But when classified according to their chemical activities these organisms form a remarkable chain whose links we are all but warranted in regarding as the varied end points of an evolutionary process. As our most primitive type we may well choose the nitrogen-fixing anaerobes of the soil. It is to Winogradsky that we turn for the first demonstration of such organisms. He named his anaerobic nitrogen-fixing organism *Clostridium Pastorianum*. Bredemann declares that nitrogen-fixation is a variable power among all organisms of the amylobacter type, and he includes under the name amylobacter, *Clostridium Pastorianum* of which bacillus he possesses a strain. These organisms are highly saccharolytic, being pectin fermenters, and they do not split gelatin. Farther up the scale we find anaerobic rods which are actively saccharolytic and do split gelatin, for example the Welch bacillus, vibrion septique, and many other forms; these organisms do not liquefy coagulated serum or produce hydrogen sulfide or other protein split products in any considerable quantity. Most of them possess no diastatic power toward pentosans, and many fail even to split pentoses. The group next in the scale are those anaerobes which, though they fail to disintegrate coagulated serum and muscle particles, are sufficiently proteolytic to free considerable quantities of hydrogen sulfide in media rich in sulfur, such as blood broth. The oedematiens type, and other less well-known organisms may be placed here. Some of these are strongly saccharolytic and others are weakly so. Further advances in attack on the protein molecule are almost invariably accompanied by a decrease in saccharolytic power. *B. aerofoetidus*, and the two strains which I term *Reglillus* are apparently slightly proteolytic, and only moderately saccharolytic. The more highly proteolytic organisms usually split monohexoses, glucose only, or no sugars at all. The most highly adapted catalytic anaerobes are those that produce both acid and alkali from their substratum in sufficient quantities to keep their hydrogen-ion end point within their optimum range and which are so highly proteolytic that they disintegrate a great variety of protein molecules and split-products. Such types are *B. histolyticus* and *B. botulinus*. Another type, known as *Bifermentans*, keeps its end point within its optimum range of growth but is not sufficiently proteolytic to continue multiplication for a long period. Such organisms are not highly but widely specialized, and are adapted to fend for themselves because they are saccharolytic as well as proteolytic.

It is to render possible a future scientific and logical classification of anaerobic organisms that this key and these generic definitions are proposed. They are manifestly incomplete, and it is certain that careful taxonomic investigations will require the emendation of many of the proposed genera. But the present paper aims to blaze a trail where meandering paths have wandered—a trail upon which a highroad may be built in the future.

I wish most heartily to thank Dr. Karl F. Meyer for many helpful suggestions which he has given me during the compilation of this classification.

Artificial Key to the Genera of the Clostridioideae

- A. Do not produce H_2S demonstrable by lead-acetate-paper test when grown in blood-broth.
  - B. Do not liquefy gelatin
    - C. Very large rods that form oval spores and store up starch in carbohydrate media.
      - Genus 1. *Clostridium* Prazmowski emmend. Heller. Type species *butyricum* as described by Winogradsky (1895).
    - CC. Rods with spherical spores.
      - D. Do not grow on media containing much protein.
        - Genus 2. *Omelianskillus* nov. gen. Type species *hydrogenicus* as described by Omeliansky (1895 and 1904b).
      - DD. Grow on ordinary anaerobe media.
        - E. Sides of the bacilli parallel, spores strictly terminal.
          - Genus 3. *Macintoshillus* nov. gen. Type species *tetanomorphus* (pseudotetanus bacillus, McIntosh (p. 32), *Bacillus tetanomorphus* Committee (p. 41)), as described by the Committee.
        - EE. Spores not always strictly terminal, sides of bacilli may not be parallel.
          - Genus 4. *Douglasillus* nov. gen. Type species *sphenoides* (*Bacillus sphenoides* Douglas Fleming and Colebrook), as described by the Committee (p. 43).
    - CCC. Slender rods with oval endspores, usually Gram-negative.
      - D. Clot milk and attack various sugars, produce much acid.
        - Genus 5. *Henrillus* nov. gen. Type species *tertius* (*Bacillus tertius* Henry) as described by Henry.
      - DD. Do not clot milk, attack few or no sugars, produce little acid.
        - Genus 6. *Flemingillus* nov. gen. Type species *cochlearius* (*Bacillus cochlearius* Douglas Fleming and Colebrook) as described by the Committee (p. 40).
    - CCCC. Gram-positive rods which are not markedly slender and which produce oval spores.
      - D. Clot milk, saccharolytic.
        - E. Sporulate meagerly, attack a few sugars; occasionally moderately pathogenic tissue invaders.

Genus 7. *Vallorillus.* nov. gen. Type species *fallax* (*Bacillus fallax* Weinberg and Séguin) as described by the Committee (p. 27).

EE. Sporulate readily, attack several sugars; not known to be pathogenic.

Genus 8. *Multifermentans* nov. gen. Type species *tenalbus* (*Bacillus multifermentans tenalbus* Stoddard) as described by Stoddard (1919 b).

DD. Do not clot milk. Large Gram-positive rods with long elipsoid spores.

Genus 9. *Hiblerillus* nov. gen. Type species *sextus* (bacillus VI of von Hibler) as described by von Hibler (1908). (Résumé by Weinberg and Séguin (p. 202).)

BB. Liquefy gelatin.

C. Produce stormy fermentation of milk and sporulate on alkaline media only.

Genus 10. *Welchillus* nov. gen. Type species *aerogenes* (*Bacillus aerogenes capsulatus* Welch and Nuttall), type 1 as defined by Simonds (1915 a and b).

CC. Do not produce stormy fermentation of milk.

D. Do not sporulate.

Genus 11. *Stoddardillus* nov. gen. Type species *egens* (*Bacillus egens* Stoddard) as described by Stoddard (1919 a).

DD. Sporulate readily.

E. Gram-positive, form woolly colonies in deep agar. Typically highly pathogenic tissue invaders of many species of animals.

Genus 12. *Rivoltillus* nov. gen. Type species *vibrion* (the vibrion septique of Pasteur) as defined in a future paper.

EE. Gram-negative, may contain Gram-positive granules. Form smooth lenticular or modified lenticular colonies in deep agar. Typically pathogenic for cattle, sheep, and guinea-pigs.

Genus 13. *Arloingillus* nov. gen. Type species *Chauvoei* (*Bacterium Chauvoei* Arloing, Cornevin and Thomas) as described in a future paper.

AA. Produce H_2S demonstrable by a lead-acetate-paper test when grown in blood broth.

B. Produce a large amount of gas from carbohydrates. Heavy Gram-positive rods with little or no tendency to sporulation.

Genus 14. *Meyerillus* nov. gen. Type species *sadowa* nov. sp. to be described in a future paper.

BB. Produce less gas from carbohydrates. Heavy Gram-positive or Gram-negative rods that form oval spores that are usually subterminal.

Genus 15. *Novillus* nov. gen. Type species *maligni* (*Bacillus oedematis maligni II*. Novy) as described by Novy.

Artificial Key to the Genera of the Putrificoideae

A. Produce an alkaline reaction in meat medium but do not grossly disintegrate the particles of meat.

B. Minute Gram-positive rods which form few spores. Clot milk. Weak tissue invaders, or non-pathogenic.

Genus 16. *Seguinillus* nov. gen. Type species *aerofoetidus* (*Bacillus aerofoetidus* Weinberg and Séguin) as described by Weinberg and Séguin (p. 161).

BB. Gram-positive rods with oval subterminal or median spores that do not greatly distend the sides of the bacilli. Do not ferment milk. Typically pathogenic tissue-invaders.

Genus 17. *Reglillus* nov. gen. Type species *progrediens* nov. sp. to be described in a future paper.

AA. Attack proteins somewhat more energetically than the above and produce a terra cotta coloration of the meat particles and soften and partially disintegrate them. (The terra-cotta color is not to be confused with the pink color produced in meat medium by the acid from sugar fermentation.) These organisms continue to multiply in meat medium at a moderate rate for months.

B. Show no blackening of the meat-particles on prolonged incubation.* Gram-negative or weakly Gram-positive rods with oval subterminal or median spores that distend the bacilli.

Genus 18. *Robertsonillus* nov. gen. Type species *primus* (Bacillus I. Hempl) as described by Hempl.

BB. Show after approximately two weeks' incubation a blackening of some of the meat-particles. Gram-negative rods with spherical end-spores. Typically produce a neuro-toxin.

Genus 19. *Nicollaierillus* nov. gen. Type species *tetani* (*Bacillus tetani* Nicollaier) to be described in a future paper.

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* "Incubation" refers to anaerobic incubation at 37°C except in the case of deep agar tubes which are incubated in air at 37°C. Meat medium is pH 7.2 to start with.

AAA. Highly proteolytic on meat medium for a short period. Produce a gray coloration or a slight blackening of the medium. Sporulate at an early stage and after three days' incubation vegetate very slowly indeed without so digesting the meat particles that they greatly decrease in size.

B. Thick rods with oval or oblong spores which are usually central and do not greatly distend the bacillus.

C. Resistant saprophytes. Occasionally invade tissue in company with other organisms or alone in debilitated individuals.

Genus 20. *Martellillus* nov. gen. Type species *bifermentans* (*Bacillus bifermentans sporogenes* Tissier and Martelly) as described by Tissier and Martelly.

CC. Delicate parasites. Gram-negative tendency.

Genus 21. *Recordillus* nov. gen. Type species *fragilis* nov. sp.

AAAA. Organisms highly proteolytic, producing on three days' incubation in meat medium partial destruction of the meat particles which continues on further incubation till the meat particles have greatly diminished in bulk. Coloration of meat usually dark brown or terracotta; blackening may or may not take place.

B. Slender rods with terminal oval spores.

C. Split sugars.

Genus 22. *Tissierillus* nov. gen. Type species *paraputrificus* (*Bacillus paraputrificus* defined by Bienstock 1906) as described by McIntosh under the name of *Bacillus putrificus* (p. 39).

CC. Do not split sugars.

Genus 23. *Putrificus* nov. gen. Type species *Bienstocki* (*Bacillus putrificus* Bienstock) as defined by Bienstock (1906)).

BB. Heavy rods with subterminal or median oval spores.

C. Do not produce balls of amino-acid crystals to a striking degree.

D. Not so highly proteolytic as organisms of following groups. Meat particles not much decreased in size, spores often larger, and more nearly spherical than in following genera. Isolated colonies in deep agar usually, but not always, large, smooth lenticular or modified lenticular structures that do not become woolly. Typically produce a powerful neuro-toxin.

Genus 24. *Ermengemillus*, nov. gen. Type species *botulinus* (*Bacillus botulinus* van Ermengem) as described by Meyer and co-workers in a future paper.

DD. Highly proteolytic, blacken meat readily, produce penetrating foul odor. Form in deep agar large woolly or spherical colonies or lenticular colonies that show a tendency to become woolly. Are not pathogenic in pure culture.

Genus 25. *Metchnikovillus* nov. gen. Type species *sporogenes* (*Bacillus sporogenes* Metchnikoff) as described by the Committee as Metchnikoff's race A (p. 36).

CC. Produce balls of amino-acid crystals in meat after a comparatively short period of incubation. Violently proteolytic, may produce an exotoxin, and invade tissue, which is vigorously digested.

Genus 26. *Weinbergillus* nov. gen. Type species *histolyticus* (*Bacillus histolyticus* Weinberg and Séguin) as described by Henry.

DEFINITIONS OF CERTAIN GENERA OF THE CLOSTRIDIOIDEAE[3]

Genus 1. *Clostridium* Prazmowski 1880, emmend. Heller. *Clostridioideae* that do not liquefy gelatin. Most species cause stormy fermentation of milk. Highly saccharolytic, many even splitting pectins. Produce considerable amounts of butyric acid as a split-product of carbohydrate fermentation. May fix nitrogen. Readily derive their nitrogen from inorganic nitrogen salts. Large rods which are frequently polymorphic and form large orgonts and oval spores. Frequently store up starch. Form in deep agar large lenticular or modified lenticular colonies. Common destroyers of plant tissue (not cellulose) in soil. Used in the retting of flax to split pectins.

Type species, *C. amylobacter* van Tieghem as described by Winogradsky (1896). Probable synonyms: *Amylobacter* Trécul, *Clostridium butyricum* Prazmowski, *Amylobakter* Gruber, *Granulobakter saccharo-butyricum* Beijerinck, *B. saccharobutyricus* von Klecki, *Granulobacillus saccharobutyricus mobilis nonliquefaciens* Grassberger and Schattenfroh, *B. amylobacter* von Hibler, *B. amylobacter* Arthur Meyer and Bredemann. Most of these are today incapable of accurate definition and several of them represent groups and not entities. For this reason the type description chosen is that of Winogradsky instead of that of Prazmowski.

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[3] For definition see Jour Bact., 6, 550.

Trécul (1865) gave the bacteria that contain starch the name of *Amylobacter*. He (1867) declared the *Amylobacter* to be heterogenetic, formed of minute particles that organize themselves into bacilli in decaying plant tissue. Van Tieghem (1877) named *Bacillus amylobacter* bacilli which contained amorphous starch during their growth stage. He believed such organisms to be the agents of cellulose destruction. Prazmowski (1880) described and figured *Clostridium butyricum*, which though it was in impure culture, was evidently of the type described above. Winogradsky (with Fribes) (1896) first defined a type that can be considered a species; he assigned no name to the organism. He declares that it does not split cellulose, but pectin. It ferments glucose, sucrose, lactose and starch in peptone media.

This type of organism has been described by many workers. It has been most thoroughly discussed by Bredemann. *Bacillus amylobacter* A. M. et Bredemann probably includes all the large starch-storing clostridia described above in the generic definition. In his investigation Bredemann used principally cytological criteria, and essential extensive chemical studies were not made. He regards all differences noted between his strains as fluctuating variations. It would seem illogical from the point of view of the general systematist, to assign merely specific rank to a group of organisms so widespread and of such abundant occurrence as are these soil anaerobes. Bredemann's investigation simply indicates that he did not find means of distinguishing his strains, or perhaps that by his technique he isolated only a restricted group of the general type. Bredemann declares that the power of fixing nitrogen varies in these organisms and cannot be used as a specific character. Bredemann's critique of his cultures is apparently very weak. Thus on page 404 he claims to have changed a Welch bacillus into an amylobacter. He quotes seriously the fantastic conceptions of Grassberger and Schattenfroh with regard to the "denaturing" of anaerobic organisms, and his conception of systematic anaerobic work is that of Lehmann and Neumann. He regards as a variant of *B. amylobacter* what appear to be coccus forms contaminating his cultures.

The chemical activities of this type of anaerobe with regard to end-products are described in some detail by Bredemann and by Grassberger and Schattenfroh. The latter find that amylase is usually present, sucrase very rarely so.

Clostridium Pastorianum is the name given to the anaerobic nitrogen-fixing bacillus discovered by Winogradsky (1896 and 1902). This author distinguishes it from other clostridia known to him by the fact that the sporangia only partially disappear from about the spores, forming what he terms a "spore capsule," and by the fact that its fermentative ability is less than is that of most soil clostridia. In peptone media it splits glucose, sucrose, laevulose, inulin, galactose and dextrin, but not lactose, arabinose, starch, rubber, mannitol, dulcitol, glycerol or calcium lactate. Presumably stormy fermentation of milk does not then take place. Obviously the production of stormy fermentation of milk, depending on the splitting of one sugar, is not to be regarded as a generic character. Grassberger and Schattenfroh found certain strains of their organism which did not attack milk with energy. *Clostridium Pastorianum* is sufficiently well differentiated by Winogradsky from the ordinary *amylobacter* or *butyricum* type to warrant its separation from that type as a separate species. Bredemann regards the "spore capsule" formation described by Winogradsky as a frequent anaerobe character. I have never seen an anaerobe strain which produced the remarkable "spore capsules" figured by Winogradsky. This author isolated *C. Pastorianum* only a few times out of many samples of earth, and he was familiar with the type usually termed *C. butyricum* or *B. amylobacter.*

Gruber distinguished two types of sporulating anaerobic granulose-storing butyric acid bacilli, of which the first is the most like the usual conception of *C. butyricum.*

Beijerinck differentiated his granulobacilli into an anaerobic and an aerobic form: Grassberger and Schattenfroh were unable to confirm this work.

Choukevitch (1911) distinguished three types of amylobacter: one fermented glucose, lactose, starch and hemicellulose; one glucose and lactose only; and a third rarely stored up starch and had little fermentative power. He (1913) identified as *B. amylobacter* strains which ferment cellulose.

Pringsheim described *C. americanum*, which fixes nitrogen less energetically than *C. Pastorianum* and ferments the same sugars as that organism, besides mannitol, glycerol and lactose. It will grow in open flasks; Bredemann terms it an anaerobe.

Douglas, Fleming and Colebrook have described under the name *B. butyricus* a medium sized bacillus that should be assigned to this genus or to *Multifermentans*. McIntosh describes what may be the same strain.

Prazmowski described under the name *Clostridium polymyxa* an aerobic organism. The generic name has since been used occasionally for aerobes. It must in future be restricted to the group of anaerobic organisms which most strongly resemble the first type described by Prazmowski, *C. butyricum*.

There apparently remains abundant critical chemical and systematic work to be done in the study of this important genus, and such work should be performed with improved technique and with cultures whose purity will stand criticism.

GENUS 2. *Omelianskillus* nov. gen.

Clostridioideae that do not grow well in media containing much protein, and may derive all their nitrogen from mineral salts. Split cellulose or hemicellulose. Do not contain starch. Long slender bacilli with spherical spores. Colonies may be produced on potato slants; they are minute, yellowish and transparent. Agents of plant putrefaction, found everywhere.

Type species *O. hydrogenicus* (the ferment of cellulose which produces hydrogen, of Omeliansky) as described by Omeliansky (1895 and 1904 b). Characters of genus. Probably several species were studied by Omeliansky. In his later work he admits that the cultures studied by him were not pure. Another type, *O. methanicus*, similar to the above, is said to be the agent of methane formation in cellulose fermentation. This species may be assigned to the same genus.

These organisms were isolated by growing them in a medium free of organic nitrogen. There may be other genera of anaerobic cellulose splitters which require some organic nitrogen for their metabolism.

Choukevitch (1911) describes under the name *B. gazogenes* an organism of active growth habit and of very strong fermentative powers, that splits starch and hemicellulose. Its morphology is similar to that of Omeliansky's organisms and it may be included in this genus.

Choukevitch (1913) considers his type 1 of *B. Rodella III* as similar to *O. methanicus* and type II as similar to *O. hydrogenicus*, but his reasons for so doing are not very sound. These two organisms do not split cellulose.

Genus 3. *Macintoshillus* nov. gen.

Clostridioideae that do not liquefy gelatin. They produce acid and gas and no putrefaction in meat media. They do not readily attack milk and they ferment few or no sugars. Gram-negative rods with parallel sides and with terminal spherical spores. Colonies in deep agar are small and irregular but not woolly. Frequently found in wounds. Apparently incapable of invading tissue.

Type species *tetanomorphus* (pseudotetanus bacillus, McIntosh (p. 32), *Bacillus tetanomorphus* Committee (p. 41)), as described by the Committee. Glucose and maltose are fermented.

Bacillus tetanoides A of Adamson (1919) is to be assigned to this group. Acid and no gas is produced from glucose and maltose by the majority of Adamson's strains, while one strain showed no fermentative ability. The former type is probably identical with McIntosh's organism.

Choukevitch (1913) describes as type 1 of the bacillus known as Rodella III a slender and highly saccharolytic organism with spherical spores, resembling morphologically Omeliansky's cellulose fermenters. It does not ferment cellulose nor liquefy gelatin and may temporarily be placed here in spite of the fact that it is said to produce hydrogen sulfide. If the organism does not liquefy gelatin it is unlikely that it produces any considerable amount of hydrogen sulfide from protein when in pure culture. This organism is a common intestinal saprophyte of cattle and sheep. A heavier bacillus, type II, is equally difficult to place. It also produces hydrogen sulfide and is highly saccharolytic.

It may be that another genus should be created for highly saccharolytic spherical end-sporing organisms that grow on ordinary media.

GENUS 4. *Douglasillus* nov. gen.

Clostridioideae that do not liquefy gelatin. They produce little gas in meat medium. They may clot milk. Gram-negative bacilli which are frequently fusiform and may show peculiar involution forms. Spherical spores are formed in the bacilli; the rods are usually widened by the spores so that their sides are not parallel. Young spores may not be truly spherical. Occasionally found in wounds. Probably incapable of invading tissue.

Type species *sphenoides* (*Bacillus sphenoides* Douglas, Fleming, and Colebrook), as described by the Committee (p. 43): the type which ferments glucose, maltose, galactose, lactose, salicin, mannitol, sucrose, dextrin and starch.

The Committee states that the fermentation reactions of this group are variable. Bacillus E of Adamson (1919) is apparently closely related to *D. sphenoides*. It produces peculiar involution forms and though it ferments several sugars (glucose, maltose, lactose and mannitol) it produces very little gas from them, and has not strong fermentative powers.

The pointed rod named *Coccobacillus proeacutus* by Tissier may be included in this genus. It does not ferment lactose or sucrose but does attack glucose.

These organisms are not to be confused with the nonsporulating anaerobic fusiform bacilli which show a spotted staining reaction and invade tissue, but grow poorly on artificial media. These latter may be trained to an aerobic habit. They have been placed in the genus *Fusiformis* in the family *Mycobacteriaceae*, order *Actinomycetales* by the Committee on Characterization and Classification of Bacterial Types, where they may well be left because of the fact that they are highly adapted parasites.

GENUS 5. *Henrillus* nov. gen.

Clostridioideae that do not liquefy gelatin. They produce acid and gas in meat medium. They clot milk readily and attack many sugars, producing much acid. Gram-negative or gram-positive slender rods with terminal oval spores. Their colonies in deep agar are large, lenticular and opaque. Very common in soil, often found in wounds, do not invade tissue.

Type species *H. tertius* (*Bacillus tertius* Henry) as described by Henry. Henry believes that the conception *B. tertius* applies to a group and not to a species. His nine strains which may be taken as a type split the monoses, bioses, mannose, xylose, starch, dextrin, glycogen, salicin, amygdalin and mannitol.

This type was described by von Hibler with the number IX. Fleming (Bac. Y), Rodella (1902) (Bac. III), Robertson (1916 a), McIntosh, Adamson (1919) and the Committee and Weinberg and Séguin also describe it. Choukevitch (1913) identifies spherical sporulating organisms with Rodella III. The original Rodella III did not clot milk and should perhaps be associated with *Flemingillus*.

GENUS 6. *Flemingillus* nov. gen.

Clostridioideae that do not liquefy gelatin. They produce little gas or acid in meat medium. They do not grow very abundantly in milk or change it. They do not show any marked tendency to split sugars. Slender Gram-negative rods with oval terminal spores. Colonies in deep agar lenticular, may show an areola of fine radiations. Frequently found in wounds, not pathogenic for guinea-pigs.

Type species *F. cochlearius* (*Bacillus cochlearius* Douglas, Fleming and Colebrook) as described by the Committee. This type is highly motile and it split none of the carbohydrates that it was grown in. It was described as bacillus III type C by McIntosh.

Bacillus C of Adamson (1919, p. 380) should be referred to this genus. It is slightly motile. McIntosh's Bacillus III A was also considered by Adamson to belong in such a group as this. It splits glucose and maltose only. We may, until the nonproteolytic slender end-sporing rods have been carefully investigated, include strains that split a few easily fermentable sugars and do not produce much acid in genus *Flemingillus*, and those that split many sugars and tolerate much acid in the genus *Henrillus*.

Choukevitch describes a number of organisms possessing a similar lack of fermentative ability to *Flemingillus*. Possibly his *B. irregularis* belongs here; *Streptobacillus anaerobicus-magnus* does not resemble this type morphologically, but does chemically.

B. ventriosus of Tissier and *B. gracilis-putidus* of Tissier and Martelly resemble this type. *Coccobacillus oviformis* and *B. capillosus* of Tissier resemble it in fermentative powers but not in morphology.

Genus 7. *Vallorillus* nov. gen.

Clostridioideae that do not liquefy gelatin. They produce gas and acid in meat medium but no digestion. They clot milk slowly and attack various sugars. Rather slender Gram-positive rods with little or no tendency to form spores. Form lenticular colonies, "coeurs jaunes," in deep agar. May invade tissue, producing oedema and gas. Pathogenicity transitory.

Type species *V. fallax* (*Bacillus fallax* Weinberg and Séguin) as described by the Committee (p. 27), the type which ferments glucose, laevulose and maltose.

Henry regards this organism as capable of fermenting many sugars and starch.

Choukevitch (1911) describes under the name *B. bifurcatus gazogenes* a large branching organism which has fermentative reactions similar to *Vallorillus*, but can hardly be included in the genus on account of its unusual morphology.

Genus 8. *Multifermentans* nov. gen.

Clostridioideae that do not liquefy gelatin. Produce gas and acid in meat medium. Clot milk readily, without stormy fermentation. Rather small Gram-positive rods with oval central or subterminal spores. Actively saccharolytic. Found occasionally in wounds, not tissue invaders.

Type species *M. tenalbus* (*Bacillus multifermentans-tenalbus* Stoddard) as described by Stoddard (1915 b). This organism, of which Dr. Stoddard was so kind as to send me a culture, does not fit into any other of the genera here defined. Stoddard's organism ferments glycerol, maltose, lactose, raffinose, glucose, sucrose, inulin, and salicin. Mannitol and dulcitol are not fermented.

Adamson describes under the name *B. butyricus* a "small or medium-sized" bacillus that ferments glucose, lactose, maltose and sucrose but not mannitol and starch. A butyric acid odor is produced. Perhaps it should be assigned to this group.

GENUS 9. *Hiblerillus* nov. gen.

Clostridioideae that do not liquefy gelatin. They do not clot milk. Large gram-positive rods which form more or less reluctantly long ellipsoid spores; they may form orgonts. Colonies in deep agar, small and lenticular or with fine radiations. May be pathogenic for the guinea-pig, producing oedema and gas, or paralysis.

Type species *H. sextus* (*Bacillus VI* of von Hibler), as described by von Hibler (résumé by Weinberg and Séguin, p. 202).

Von Hibler describes another species which he terms VII. These organisms have much in common and we are probably justified in including them in one genus as *Hiblerillus sextus* and *Hiblerillus septimus*. The latter resembles an organism described by Tizzoni and Cattani, according to von Hibler. There are probably many organisms in soil which are pathogenic for rabbits and guinea-pigs when given certain conditions favorable to invasion, which rarely invade under natural conditions or which on account of shy growth habit are missed when they invade in the company of other organisms.

To this genus may be assigned two organisms isolated from the intestine of the horse and described by Choukevitch (1911); they possess similar fermentative ability: the production of acid and no gas in glucose agar. A non-pathogenic one, *Streptobacillus anaerobicus-rectus* may be termed *H. rectus*; another, *H. megalosporus*, produced a fibrino-purulent peritonitis in a guinea-pig.

GENUS 10. *Welchillus* nov. gen.

Clostridioideae that liquefy gelatin but do not produce hydrogen sulfid demonstrable by lead-acetate-paper test in blood-broth. They produce much acid and gas on meat medium, but they do not digest it, nor do they digest casein, coagulated serum, or eggwhite. They produce stormy fermentation of milk and attack many sugars vigorously. Their multiplication is extraordinarily rapid; they are killed by their own growth products in acid media, in which they fail to sporulate. Deeply Gram-positive non-flagellate rods with square ends. They produce oval subterminal or median spores in alkaline media, or in media free of fermentable carbohydrate; these spores do not bulge the sides of the bacilli. When growing rapidly the rods are very short, and resemble closely no other type here listed except *Stoddardillus*. When growing slowly the rods are less abundant and longer, and may be mistaken for *Martellillus* and similar organisms. Typically intestinal saprophytes; ubiquitous. Many strains produce toxin and invade tissue, forming gas, and causing the formation of oedema, and in many cases causing the disintegration of muscle and of connective tissue without the production of a foul odor. This disintegration occurs only *in vivo*, and is probably due to the enzymes of the host tissue. *Welchillus* are the most frequent anaerobic invaders noted on the hospital autopsy table: the organisms are present in human intestines. Causative agents of a probably greatly overestimated percentage of gas gangrene cases following war wounds. Comparatively rare as animal invaders.

Type species *W. aerogenes* (*Bacillus aerogenes-capsulatus* Welch and Nuttall) type I as defined by Simonds (type IV of Esty). Ferments, besides other carbohydrates, inulin and glycerol. Usually pathogenic for guinea-pigs.

Synonyms. *B. phlegmones-emphysematoseae* Fraenkel, *B. perfringens* Veillon and Zuber, 'Bacille de reumatisme aigue' of Achalme, *Butyribacillus immobilis-liquefaciens* Grassberger and Schattenfroh. Descriptions and discussions, most of them with large bibliographies, are to be found under the names of the following authors: von Hibler, Simonds, Robertson, Weinberg and Séguin, Henry, McIntosh, Adamson, The Committee, Jablons, Esty. Simonds divided the group into four sub-groups on the basis of the fermentation of inulin and glycerol. Henry and Esty substantiated this finding. The latter finds his strains divisible into two sub-groups on the basis of the sensitiveness of the spores to heat. The division thus made does not coincide with those secured by means of sugar fermentation. McIntosh admits a species which sporulates on ordinary media. The Committee, on which McIntosh later served, do not mention such a type. The chemical behavior of *Welchillus* has most recently been studied by Wolf (1919) and Wolf and Harris (1917, a, b, and c). Agglutinins are extremely difficult to produce with these organisms, and are found to agglutinate only homologous strains. Werner succeeded in agglutinating one non-homologous strain. Robertson (1916 b) failed to immunize guinea-pigs with bacterial protein. Toxins, according to the work of Bull and Pritchett, are all neutralized by the same antitoxin. Esty immunized guinea-pigs with young whole cultures.

GENUS 11. *Stoddardillus* nov. gen.

Clostridioideae of energetic growth habit that liquefy gelatin, but do not produce H_2S demonstrable by a lead-acetate-paper test in blood broth. Produce abundant gas but little acid in meat medium. Grow very shyly or not at all in milk. Attack a few sugars, but do not produce much acid. Short chunky Gram-positive rods which do not form spores. Colonies in deep agar large, lenticular and opaque. Not easily distinguished from *Welchillus*. May invade tissue, causing considerable destruction of muscle but no foul odor. One strain, reported from a case of human gas gangrene.

Type species *S. egens* (*Bacillus egens* Stoddard) as described by Stoddard (1919 a). Splits glucose, laevulose, mannose, maltose, dextrin, glycogen, inosite and glycerol. Does not sporulate on six days' incubation in inspissated serum.

Such organisms as this are probably not nearly so rare as reports would indicate because they do not sporulate and are killed in most anaerobic isolation procedures. Because of their close resemblance to *Welchillus* and because of the ubiquity of organisms of that genus their detection is rendered still more difficult.

It may be that later workers will prefer to include this organism in the genus *Welchillus*. The action on milk is dependent on the fermentation of lactose, and sugar fermentations are not to be regarded as of generic significance. I place it in a genus by itself because spore formation has not been demonstrated for this organism.

GENUS 12. *Rivoltillus* nov. gen.

Clostridioideae possessing moderately strong saccharolytic powers. Liquefy gelatin but do not produce H_2S demonstrable by a lead-acetate-paper test in blood broth. Produce in meat medium gas and a pink coloration which does not rapidly fade. Clot milk. Do not liquefy serum or egg or disintegrate meat particles. Gram-positive rods, usually short, with median, sub-terminal, or terminal spores, which usually bulge the sides of the bacillus. Sporangia not often much larger than vegetative rods. In tissue the sporangia may be uneven in their staining reactions, "granulose" being present; orgonts are long, frequently with parallel sides. Usually form chains on the liver of animals. Colonies in deep agar, though they may start as lenticular structures, consist later of a dense center and a wide loose woolly periphery. They vary in size, etc., according to species. Typically highly pathogenic tissue invaders that produce haemolysis and gas in the animal body. Pathogenic for a wide range of species.

Type species *R. vibrion* (the vibrion septique of Pasteur), as defined in a future paper. Robertson (1920) has divided the group into four sub-groups on the basis of the agglutination reaction.

Probably the group of anaerobes whose nature is most frequently discussed. The morphological resemblance of individuals and of colonies to organisms of the sporogenes type led to the frequent description of mixed cultures of the two types, usually under the name *B. oedematis-maligni.* The literature on this group is too extensive to quote. See Ghon and Sachs, Meyer, Weinberg and Séguin, Robertson, the Committee, Wolf (1918). A review of the animal infections has been made by Heller (1920). Under the name *Bacillus tumefaciens* (not to be confused with the plant pathogen *Bacterium tumefaciens*). Wilson describes an organism similar to those of this genus. His description does not convince the reader as to the purity of the culture studied: a mixture of vibrion septique and oedematiens-like organisms would behave as did Wilson's bacillus.

GENUS 13. *Arloingillus* nov. gen.

Clostridioideae that attack sugars with considerable energy but have a somewhat restricted action on proteins. Liquefy gelatin but do not produce H_2S demonstrable by a lead-acetate-paper test in blood broth. In meat medium produce gas and a pink coloration that soon fades. Autoagglutinate readily. Clot milk if blood is present. Do not digest serum or egg. Vegetative forms are small gram-negative rods with even staining; forms about to sporulate are uneven in staining reaction, often far larger than vegetative rods, citron or spindle shaped; orgonts (see Heller), show marked tendency to store up granulose. Spores oval, may vary greatly in length. Bacilli do not form long chains on the liver of animals. Colonies in deep agar lenticular, sometimes showing concentric formation, or compound lenticular. Colonies vary considerably according to species. Typically toxic tissue invaders which produce marked haemolysis. Pathogenic for guinea-pigs, cattle and sheep.

Type species *A. Chauvoei* (*Bacterium Chauvoei* Arloing, Cornevin, and Thomas) as described in a future paper.

This genus contains several species which will be discussed. These organisms show some similarity to those of the genus *Clostridium.*

The *B. enteritidis-sporogenes* Klein, as described by von Hibler (1908) should probably be included in the genus. The only character which is markedly different from that of the genus is the energetic fermentation of milk shown by von Hibler's bacillus IV.

GENUS 14. *Meyerillus* nov. gen.

Clostridioideae that produce H_2S on blood broth and liquefy gelatin. In meat medium they produce gas but little or no pink coloration; they show no marked proteolytic action. Do not readily attack milk. Large Gram-positive rods with little or no inclination to form spores. Attack a few sugars. Colonies in deep agar large, opaque and lenticular. Typically tissue invaders of marked power, attacking and digesting *in vivo* the connective tissue more than the muscle.

Type species *M. sadowa* nov. sp. To be described in a future paper. This organism was at first taken for *B. Welchii.* It does not sporulate. It is one of the four guinea-pig invaders isolated from a case of human gas gangrene. I am inclined to place the sporulating bacillus L of Adamson (1919) in this genus but have not handled that organism. Perhaps it is premature to decide whether sporulation may be used as a generic character. Adamson finds bacillus L very slightly proteolytic on milk and not so on other media. *M. sadowa* does not grow on milk.

GENUS 15. *Novillus* nov. gen.

Clostridioideae that in blood broth produce H_2S demonstrable by lead-acetate-paper test; they liquefy gelatin. Produce gas and on continued incubation produce a pink coloration in meat, but this color rapidly fades. Autoagglutinate with extreme readiness. Slowly attack milk and a few carbohydrates, but do not form much acid. Heavy rods of apparently shy growth habit on most media; form a few oval spores that may or may not bulge the sides of the bacilli. Colonies usually large and opaque, may form slender projections or even long fine woolly filaments. Frequently yellowish. Typically toxic tissue invaders which frequently cause the formation of a thick gelatinous oedema that does not lose its gelatinous consistency on section. May also produce gas and a black haemorrhagic condition of the muscle if rapid invasion by large numbers of bacilli takes place. One strain causes considerable tissue destruction. Pathogenic for guinea-pigs, mice, man, hogs, horses, cattle.

Type species *N. maligni* (*Bacillus oedematis-maligni II*, Novy) as described by Novy.

The lesions typically produced by organisms of this group were first described by Koch. Gohn and Mucha give an elaborate description of an organism which belongs to this genus. Von Hibler studied four strains of "Novy's bacillus." Descriptions of organisms of this type have been given by Kerry, by Rivas and by Diedrichs. Weinberg and Séguin described *B. oedematiens* which is different from *N. maligni* but is to be included in this genus. *B. bellonensis* Saquépée also belongs in this genus, as may also the organism called by Adamson bacillus S (1919, p. 373). I have found a type that was rapidly fatal to horses, and another different somewhat from all the rest in a septic wound, and hope to define these species accurately in another paper. Wolf (1920) has studied the biochemistry of an organism of this group.

DEFINITIONS OF CERTAIN GENERA OF THE PUTRIFICOIDEAE[4]

GENUS 16. *Seguinillus* nov. gen.

Putrificoideae that do not exert a marked action on protein. Soften and may blacken meat. Milk clotted, may later be digested. Attack a few sugars. Very reluctant to form spores. Minute Gram-positive or Gram-negative rods, uniform in thickness but not in length. Spores oval, sub-terminal. Deep colonies lenticular or modified lenticular. Occasional tissue invaders which produce a proteolytic gangrene or phlegmon.

Type species *S. aerofoetidus* (*Bacillus aerofoetidus* Weinberg and Séguin) as described by Weinberg and Séguin (p. 161). The organism forms oval subterminal spores.

These may well be called "borderline" organisms. Henry places them in his "saccharolytic group" on account of their sugar-splitting proclivities. A strain given by me by Weinberg does not grossly attack meat particles.

GENUS 17. *Reglillus* nov. gen.

Putrificoideae that show to the eye little proteolytic action on meat. They may or may not blacken it slightly, but the meat particles do not diminish in size. Sugars are attacked. Milk is digested. Gram-positive rods which readily form oval subterminal or median spores. Colonies minute, opaque, complex, yellow. Typically toxic tissue invaders which produce in the guinea-pig a clear white oedema that does not rapidly lose its oedematous nature on section.

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[4] For definition see Jour. Bact., 6, 550.

Type species *R. progrediens* nov. sp. To be described in a future paper. Two species, one found in a case of human gas gangrene (see Barney and Heller), the other in a sheep.

GENUS 18. *Robertsonillus* nov. gen.

Putrificoideae that produce on meat medium a little gas and a terra cotta coloration, multiplying slowly for a long period. A black pigment is absent. Sugars not fermented. Weakly Gram-positive or Gram-negative rods with oval spores, usually subterminal, that somewhat distend the bacillus. Two spores often occur in one rod. In old cultures enormous snakey Gram-negative rods are produced. One species, found twice in wounds. Non-pathogenic for guinea-pigs in pure culture.

Type species *R. primus* (Bacillus I, Hempl) as described by Hempl. Descriptions of bacilli similar to this have not been noted. Proteolytic organisms producing a terra cotta coloration in meat medium are not uncommon, however.

GENUS 19. *Nicolaierillus* nov. gen.

Putrificoideae that in meat medium produce gas and various color changes: yellowish, pink, grey or mauve, depending on the medium; the particles of meat are gradually suffused with a black pigment, and bleach at the top. The meat is softened but the particles do not greatly diminish in size. Do not attack sugars. Gram-negative (weak methyl violet) rods that form terminal spherical spores. Colonies in deep agar diverse. Common in soil, found in horse feces, may multiply in wounds, but do not normally invade tissue. Produce a characteristic neuro-toxin.

Type species *N. tetani* (*Bacillus tetani* Nicolaier), to be described in a future paper.

Tulloch (1917 and 1919) has divided the group into four types on the basis of the agglutination reaction. Adamson (1920) describes the cultural behavior of five strains of *B. tetani.* Two papers on the behavior of my cultures are forthcoming.

This is not the genus *Plectridium* of Fischer. *Plectridium* included some butyric acid bacteria, the tetanus bacillus, a putrefactive organism, *Plectridium putrificum*, and other genera not yet known; and it embraces, according to my scheme of classification, elements altogether incoherent. Were the descriptions fuller or were I better acquainted with the proteolytic group, two more genera of proteolytic spherical end-sporers might be suggested: Those that blacken meat readily, e.g., *B. cadaveris-sporogenes*, Klein; and those which do not blacken it at all, e.g., *B. tetanoides B* of Adamson.

GENUS 20. *Martellillus* nov. gen.

Hardy *Putrificoideae* that in meat medium multiply rapidly at an early stage of incubation, producing a greyish coloration and later a blackish deposit on the meat particles, and after three days' incubation cease to multiply actively. Sporulate early in the development of a culture, later cease to do so but vegetate very slowly. Produce very little gas in meat medium. Digest milk. Attack a few sugars. Heavy deeply gram-positive rods, may vary greatly in size. Spores usually cocoon-shaped, usually median or sub-terminal, do not greatly bulge the sides of the bacillus. Colonies in deep agar lenticular, irregular, or stellate. Common putrefactive organisms that readily invade tissue in company with other organisms, producing a greenish proteolytic gangrene. I have found them in the heart's blood and organs of a woman dying of pernicious vomiting and uraemic poisoning and apparently the only invader in a mouse dying from an otherwise unknown cause. The Committee find *B. bifermentans* in acute cases of gas gangrene. Weinberg and Séguin state that such an organism invades guinea-pigs in company with *B. perfringens.* It is possible that *Martellillus* bacilli produce metabolites poisonous to themselves and to animals and that cause early sporulation. They frequently cease active multiplication when the culture has a reaction near pH 7.0.

Type species *M. bifermentans* (*Bacillus bifermentans-sporogenes* Tissier and Martelly) as defined by Tissier and Martelly.

Hempl has described a similar organism not identical with that of Tissier and Martelly (Organism II, which may be renamed *Martellillus proteolyticus*). Bacillus II of Choukevitch probably belongs in this genus, as does von Hibler's bacillus XV. *B. sporogenes B*, as described by Choukevitch also belongs here. A rather shyly growing species sent me by Major Nichols which was named *B. bellonensis* Saquépée, was apparently pure, non-pathogenic, and referable to this genus. It was not the organism described by Saquepee as *B. bellonensis*, nor did it resemble other strains sent me by Saquépée. Adamson describes two species of this type. One under the name of *B. bifermentans-sporogenes*, which is non-motile and does not split sugars, the other under the name of "Central spore bacillus" which he identifies as McIntosh's type XII, which is motile and splits glucose and maltose. The type species is, however, supposed to be saccharolytic. McIntosh's type XII (*parasporogenes*) does not behave in my hands as does this latter type of Adamson's and I should not include them in the same genus. McIntosh's type XIII, a pathogenic proteolytic organism, should probably be placed here. Some of the organisms here listed are probably identical, but one is not warranted in so considering them without a direct comparison of strains. Study of many strains would probably necessitate the division of this genus into two, on the basis of carbohydrate fermentation.

This is one of the important groups that most need systematic investigation.

GENUS 21. *Recordillus* nov. gen.

Putrificoideae that, though they sporulate, are exceedingly delicate and soon die in meat medium and other media. Their growth in meat medium resembles that of the organisms of genus *Martellillus;* they do not produce much gas, they color the meat particles a greyish color and form a blackish pigment. Gram-negative rods with central or sub-terminal cocoon-shaped spores. Parasitic forms which infect cattle in California and Nevada. I venture to place such organisms in a separate genus on account of their parasitic habit and on account of their delicacy. It may be that they are descended from organisms of the genus *Martellillus.*

Type species *R. fragilis* nov. sp. Characters of genus.

I regret that the strain kindly sent me by Dr. Records died soon after its arrival. As it had sporulated in meat medium this was surprising, but was consistent with Dr. Records' description of the behavior of the organism. The strain was isolated from a liver infarct in a cow.

Genus 22. *Tissierillus* nov. gen.

Putrificoideae that attack sugars and clot milk. Slender Gram-negative or Gram-positive rods with oval terminal spores. Colonies in deep agar have radiate periphery and opaque center. Frequently intestinal saprophytes.

Type species *T. paraputrificus* (*Bacillus paraputrificus* defined by Bienstock) as described by McIntosh under the name *Bacillus putrificus.* Ferments glucose, maltose, lactose, sucrose and starch.

References to organisms of this type are made by Passini, Moro, Metchnikoff and Kligler. Very likely some descriptions of this type were based on the behavior of mixed cultures.

Genus 23. *Putrificus* nov. gen.

Putrificoideae that do not attack sugars. Slender Gram-negative or Gram-positive rods with oval terminal spores. Colonies in deep agar have radiate periphery and opaque center. Putrefactive organisms found in soil and wounds.

Type species *P. Bienstocki* (*Bacillus putrificus* Bienstock) as defined by Bienstock (1906). References: Bienstock (1884, 1901, 1906), Klein, Rodella (1905). The latter differentiated three types of what he termed *B. putrificus.* Tizzoni, Catani and Baquis described two organisms of this general type. *B. postumus* of Würcker is of this group. His *B. putrificus* is of sporogenes affinities. I possess a strain (IOR) isolated by Miss Robertson or myself from a wound, which is mildly proteolytic, does not split sugars, is Gram-negative, and on serum produces oval terminal spores. Its deep colonies are transparent, lenticular, with a protruding fluff. Dr. Meyer tells me that he occasionally encounters such organisms, very slow in initial activity in pure culture, but more active in mixed culture, mildly proteolytic in their action on meat. The colony is not unlike that of *B. botulinus*.

The Committee find that many so-called "*Putrificus*" strains consist of a non-proteolytic oval end-sporer contaminated with a sporogenes-like organism. In the recent German literature "*Putrificus*" refers usually to the sporogenes type. Metchnikoff's strains termed *Putrificus* do not resemble closely those described by Bienstock.

GENUS 24. *Ermengemillus* nov. gen.

Putrificoideae that produce a yellowish coloration in meat medium, and later blacken and digest it. They are more highly proteolytic than the organisms of the foregoing genera. They ferment various sugars. Gram-positive rods which form sub-terminal oval spores. Their colonies in deep agar are, when discrete, lenticular or kidney shaped, and may show tufted smooth or woolly polar projections and infrequently fine loose woolly radiations. Fairly common in soil, grow readily in vegetable material and on meat. Form a characteristic and powerful neuro-toxin.

Type species *E. botulinus* (*Bacillus botulinus* van Ermengem) as described by K. F. Meyer and co-workers in a future paper.

Leuchs discovered that there were two types of toxin produced by different European strains. Burke found two types of toxin produced by different American strains. Probably a number of species may be distinguished by careful methods.

McIntosh and the Committee find that *B. botulinus* shows slight proteolytic power, and meat is stated to be a poor medium for the growth of the organism. My experience has been very different from theirs. Indubitably pure cultures of *Ermengemillus* grow readily on meat medium (pH 7.2) and may show obvious putrefaction in a few days when incubated anaerobically at 37°. But the meat particles are not rapidly diminished in size by the proteolytic action. The observers who did not note the proteolytic action of these organisms on meat must have used an acid meat medium, or a substratum poor in peptones and peptids, or must have incubated their cultures at room temperature. Von Hibler found that the organism blackened brain medium.

GENUS 25. *Metchnikovillus* nov. gen.

Highly proteolytic *Putrificoideae* that readily blacken meat. They do not produce in it abundant amino-acid crystals, but digest meat, serum, egg and casein rapidly, forming more alkali or less fatty acid than do the organisms of the succeeding group. They split few sugars. Gram-positive or weakly Gram-positive rods, vegetative forms uniform and considerably smaller than sporangia. Sporulate readily in ordinary media, forming oval spores which are usually sub-terminal, though in some strains median spores predominate. Multiplication is exceedingly active, forty-eight hour colonies in deep agar are large and woolly. Frequently a few colonies are larger than the others but they do not give rise in a following generation to a preponderating number of large colonies. Ubiquitous. Common intestinal organisms, abundant in soil; very common in infected wounds. Not capable of invading in pure culture in moderate doses, may invade in company with other organisms or alone when given in large doses.

Type species *M. sporogenes* (*Bacillus sporogenes type A* of Metchnikoff) as described by the Committee (p. 36). Klein described as *B. enteritidis-sporogenes* a mixed culture which contained a non-proteolytic organism, a pure culture of which was described by von Hibler as *B. enteritidis-sporogenes* Klein (von Hibler IV). This tissue invading pathogen, thought by some to have been *B. Welchii,* is most nearly referable to the genus *Arloingillus.* The strain was derived from patients with enteritis and it was apparently contaminated with a proteolytic organism of the genus *Metchnikovillus.* Metchnikoff described two types, A and B, of intestinal anaerobes which he thought were similar to the organism of Klein, and which he termed *B. sporogenes.* His descriptions permit of no identifications. Choukevitch, working in Metchnikoff's laboratory described *B. sporogenes* A and B more carefully. Type B should be referred to genus

Martellillus. Weinberg made the identification of type A more exact, and the Committee discuss it at some length. They allow two species, *B. sporogenes* type A of Metchnikoff, and *B. parasporogenes* McIntosh, which are different in colony formation and serologically. Donaldson describes the "Reading" bacillus, to be assigned to this genus. Superficial acquaintance with many strains of proteolytic anaerobes leads me to suggest that *Metchnikovillus* may be defined as a genus of many species; Dr. K. F. Meyer is also of this opinion. Henry (p. 361) believes it likely that the conception "sporogenes" refers to a group of organisms.

Barger and Dale, Weinberg and Séguin, M. E. Bullock and the Committee discuss the poisonous growth product of this type of organism: it is apparently a lower split-product than are true toxins. Some of these authors find that the culture filtrate of *B. sporogenes* increases the toxicity and invasive power of *B. Welchii*. Donaldson and Joyce placed the Reading bacillus in wounds to digest necrosed muscle and report no accidents due to its presence. Wolf (1919), Wolf and Telfer, and Wolf and Harris (1917, a and b) describe the chemical activities of these and related organisms.

Because of their universal occurrence and active growth habits, organisms of this type frequently contaminate anaerobic cultures. Their chemical activities are thus described in conjunction with the pathogenic properties of the cultures in which they are active. The descriptions which tally more or less accurately with the sporogenes type are legion. Thus *B. oedematis maligni* Koch of von Hibler was probably a mixture of an organism of this genus with a chain-forming vibrion septique; while bacillus XI of von Hibler (1908) was probably such a mixture with a similar pathogen which formed chains somewhat reluctantly (Pl. II, fig. 3). I am today able to discover only the sporogenes type in von Hibler's strain of bacillus XI. Whether bacillus XIX of McIntosh should be included in the genus *Metchnikovillus* is difficult to state. It is apparently an active tissue invader and forms smooth lenticular colonies.

When surface methods of isolation are followed this type of organism is very difficult to remove from cultures of other anaerobes. The Committee emphasize this point. I have had no more contaminations by sporogenes than by aerobes and by various other anaerobes. The organism is common in nature and gains entrance to pure cultures occasionally.

GENUS 26. *Weinbergillus* nov. gen.

Highly proteolytic *Putrificoideae* that in meat medium cause the formation of balls of amino-acid crystals. They digest the meat particles till their bulk is greatly reduced and form large amounts of ammonia, amino-nitrogen and fatty acids. They digest the casein in milk rapidly. Attack few or no sugars. Produce little or no gas in agar media. Gram-positive or Gram-negative rods with sub-terminal oval spores. Colonies in deep agar small, delicate woolly structures. May invade living tissue in company with other organisms, or at times alone, producing a rapid and complete digestion of muscular and connective tissue structures.

Type species *W. histolyticus* (*Bacillus histolyticus* Weinberg and Séguin) as described by Henry (p. 370). Weinberg and Séguin and the Committee allow considerable variation in characters for the strains termed by them *Bacillus histolyticus*.

It is possible that *B. sporogenes-parvus* of Choukevitch may belong in this genus. Wolf and Harris (1918) have made a chemical study of what is probably the type strain—they received it from Henry who received it from Weinberg.

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No. [illegible]

Reprinted from
THE JOURNAL OF INFECTIOUS DISEASES, Vol. 30, No. 1, Jan., 1922, pp. 18-32

NOTES ON THE GENUS NICOLAIERILLUS (B. TETANI)

STUDIES ON PATHOGENIC ANAEROBES VII

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As one searches through the literature on the tetanus bacillus he is forcibly struck by its meagerness. The biology of the organism itself has been largely neglected because of the interest centering around the powerful toxin that it produces, and around the fascinating mechanism governing the distribution of the toxin in the nervous system. In the history of few diseases have individual case reports been so frequently detailed; in few diseases known to be infectious has the biology of the causative organism been so little studied. Yet the pathogenicity of the bacillus depends on its ability to live and multiply just as much as on its toxin production.

This paper gives a few notes on the behavior of certain tetanus strains that have found their way to this laboratory. The study of the tetanus organisms was not undertaken as other than a side line during the investigation of tissue invading pathogens. These notes make no pretense of being in any way exhaustive, and are intended simply to serve as an introduction to several papers by myself and other workers who have studied the same strains.

The anaerobic bacilli that cause tetanus have long been referred to one species under the name Bacillus tetani. In a recent classification[1] it was pointed out that such anaerobic bacilli as behaved similarly on ordinary mediums and had similar morphology should be placed in genera rather than in species because in such groups it is usually possible to demonstrate common types that differ consistently in minor characters and correspond to the general taxonomist's idea of species. Systematic bacteriology is so little developed that it is at present very difficult to find characters that are acceptable for the differentiation of strains. Especially is this true in the groups of anaerobic rods. One finds that a type of character that is apparently consistent in one genus is subject to variation in another and for each group various

Received for publication, Aug. 18, 1921.

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[1] Heller, H. H.: Jour. Bacteriol., 1921, 6, p. 521.

characters must be studied before a logical classification can be formulated. Such study cannot be attempted for many genera by any one investigator. Certain points of interest bearing on the classification of the tetanus bacilli are deserving of publication.

The genus Nicolaierillus was defined[2] as follows:

> "Putrificoideae that in meat medium produce gas and various color changes: yellowish, pink, grey or mauve, dependent on the medium; the particles of meat are gradually suffused with a black pigment, and bleach at the top. The meat is softened but the particles do not greatly diminish in size. Do not attack sugars. Gram-negative (weak methyl violet) rods that form terminal spherical spores. Colonies in deep agar diverse. Common in soil, found in horse feces, may multiply in wounds, but do not normally invade tissue. Produce a characteristic neuro-toxin."

The known strains referable to this genus are probably all toxigenic because it is the toxigenic phenomenon that usually leads to their isolation. Other anaerobes of similar morphology have been isolated; they are nontoxigenic and are culturally referable to other genera. The organisms producing the neurotoxin that causes clinical tetanus are apparently all referable to the genus Nicolaierillus. Nevertheless, it is probably unwise to restrict the generic definition to such strains as are toxigenic; many times difficulty is experienced in obtaining toxin from known toxigenic strains, and the nature of toxin and the process of toxin formation are too little understood for us to use the production of a poison as a critical generic character.

The Tetanus Committee, under the chairmanship of Sir David Bruce, determined, in 1916, to investigate the cause of the occurrence of cases of tetanus that proved refractory to tetanus antitoxin. Tulloch undertook a serologic investigation of the strains of B. tetani obtainable from laboratories and isolable from septic wounds. In three reports[3] he published his results. He succeeded in demonstrating that the agglutination reaction separated the group into four distinct types. The toxins produced by these types were apparently identical. Agressins were produced that were relatively specific to each type and were apparently unfiltrable or highly labile.

COLONY FORMATION OF NICOLAIERILLUS STRAINS—MUTATION

A search for descriptions of tetanus colonies in deep agar is not very illuminating. Kitasato[4] pictures a deep colony, apparently in gelatine, that is a very dense symmetrical sphere with encircling fine short radiations. He states that old colonies consist of thread-like masses. Kitasato is credited with being the first to isolate the tetanus bacillus in pure culture. This he probably did, but his critique of the purity of a tetanus culture was not reliable, for the cut accompanying the description of a pure culture shows two typical sporulating rods of undeniable sporogenes type. I have never found any such subterminal oval sporulating organism in a pure tetanus culture.

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[2] Heller, H. H.: Ibid., 1922, 7, Jan.

[3] J, Roy. Army Med. Corps, 1917, 29, p. 631; Proc. Roy. Soc., B, 1919, 90, p. 145 and p. 529.

[4] Ztschr. f. Hyg. u. Infectionskrankh., 1889, 7, p. 225.

Apparently the work of Sanchez-Toledo and Veillon[5] was careful. They describe agar colonies, as well as gelatine colonies, finding the agar colonies the less characteristic. The "clouds" are less transparent, and the "needles" less fine. Their pictures of deep gelatine colonies may have been of pure cultures and may not. The cuts (lithographs) leave some room for doubt as to whether a slender oval endsporulating organism may not have been present, but the sporogenes type is apparently absent.

Fränkel and Pfeiffer,[6] in 1892, depicted a dilution shake of tetanus colonies in deep gelatine, and this has been extensively copied in textbooks. Apparently two forms were present. Were the culture one of another anaerobe than the tetanus bacillus, one would be justified in concluding that a mixed culture had been studied. But colonies of a mutated tetanus strain may well have been the subject of the photograph.

Sanfelice[7] (p. 356) states that gelatine colonies are not characteristic, but that the deep agar ones may be distinguished from those of other anaerobes by the fineness of their filaments; he pictures two sorts of deep agar colonies (Figs. 41 and 42).

Righi[8] found a fluffy colony with a dense nucleus characteristic for tetanus organisms, in spite of the serious contamination of his cultures. Grixoni[9] recognized the same type.

Von Hibler's[10] critique of the purity of tetanus cultures was exceedingly at fault. Strain 14 (pl. II, fig. 15) shows only clostridial forms with oval spores, and strain 15 (fig. 16) shows two thirds of the bacilli of sporogenes type and one third of tetanus type. Von Hibler tells us of the behavior of strain 11, that was long grown on glycogen-containing serum medium. Many bacilli thereafter "lost" the power of liquefying gelatine, but regained it after growing six days in the muscles of a living guinea-pig. Von Hibler makes many interesting statements on atypical colonies, on injuring of the bacterial protoplasm by growing the organisms on unfavorable mediums, etc. Some of his observations may refer to real mutations, but in view of his astounding ignorance of the morphology of tetanus bacilli his notes cannot be considered of value. It is of interest that von Hibler's critique of the purity of non-proteolytic anaerobic cultures was, in the main, fair, while the distinctions between one proteolytic type and another were not duly appreciated. Von Hibler states that deep tetanus colonies in gelatine are spherical with radiations, and a concentric space of liquefaction develops within them. In agar deep colonies are "zerschlissen" or of rough contour. A cut (pl. 3, fig. 6) shows an irregular woolly colony. Atypical ones pictured (pl. 4, figs. 2, 3 and 4) are lenticular with woolly projections.

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[5] Arch. de méd. expér., 1890, 2, p. 709.

[6] Mikrogr. Atlas d. Bakterienkunde, 1890, plates 27-41.

[7] Ztschr. f. Hyg. u. Infektionskrankh., 1893, 14, p. 339.

[8] Riforma med., 1894, 3, p. 651.

[9] Ibid., p. 698.

[10] Ueber di path. Anaeroben, 1908.

McIntosh [11] states that "Shake colonies are best obtained in glucose agar; in the medium the colonies in twenty-four hours are relatively large, and at the end of forty-eight hours may measure 2-3 mm. in diameter. The growth is delicate and forms a light, cloudy mass consisting of very delicate intermingled filaments, spreading out from a small central nucleus." McIntosh pictures two colonies that do not look alike. The Medical Research Committee [12] give a similar account of tetanus colonies in deep agar.

Textbooks quote Kitasato's original description almost verbatim. These descriptions of deep and surface gelatine colonies are not readily applicable to our present day technic that employs agar almost exclusively. Yet one modern textbook after another gives us solely observations dating back to 1889. Von Lingelsheim,[13] in an elaborate compilation, apparently wonders whether a deep agar tetanus colony may be distinctive in morphology, but leaves the matter for the future to decide.

While anaerobes of most groups or genera make colonies of somewhat similar structure, the strains of this genus form colonies of exceedingly diverse types. A series of these is illustrated by the photographs accompanying this article. Of interest is the extreme variation noticed in the size of the colonies produced by the different strains. The range in size of colonies of 24 hours is from invisible spots 0.02 mm. in diameter to huge fluffs 3 mm. in diameter. Form also is diverse. The slow-growing types are apparently nonmotile and form colonies of fundamentally lenticular contour. These lenticular colonies are complex and often form peculiar lengthened structures (plate 1). Such shapes are unusual in other groups of anaerobes. The large colonies owe their size to higher motility and to greater powers of utilization of their substratum as food.

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[11] Med. Research Committee, Special Report Series 12, 1917.

[12] Ibid., 39, 1919.

[13] Kolle u. Wassermann: Handbuch d. path. Mikroorg., 1912, 4, p. 737.

The colony forms illustrated may be loosely divided into several types. These types should probably not be termed species. A relationship is not necessarily implied between the various strains that form each type of colony. A similar utilization of nutriment and a similar motility are all that connect the strains that form colonies resembling one another. Thus strains that agglutinate differently may make colonies of the same type, although the agglutination reaction is probably much more fundamentally specific than is the shape of the colonies.

Neither do the differences between these colonies represent, in the majority of cases, what is usually termed variation. When the variations for a given strain are once understood, it is found that mutations occur that in turn breed true, and may be sharply distinguished from variations. The colony form becomes the chief expression of any mutation that may take place in the metabolism of the organism. De Vries[14] employed specific and not subspecific names for what he supposed to be the products of single mutations in the genus Oenothera. These later proved to be recombinations representing sums of single mutations that had occurred previously, any one of which would not deserve specific differentiation. In other groups such as the insects, whose mutations have been well studied, a trinomial nomenclature is resorted to, and the specific name of the original wild type has been retained, while a Latin or vernacular name has been applied to the mutant, which is termed a biotype, the name subspecies being reserved for groups that are delimited by their geographic distribution. Biotype, then, is a name that may be used for these elementary species that differ from one another and breed true, but we should not term two similar colony forms of different history the same biotype, as one might do with two strains of similar higher forms developed from two different types of parents, for the reason that the colony character is not so well understood as are the characters of higher plants and animals, is not so significant or so truly an essential part of the organism as are the leaves or stems of complex forms, and there is more chance for a colony type to represent various elementary species than in the case of more elaborately detailed characters. But we should expect that different strains and species of our organisms, like those of more complex forms, should frequently mutate independently in the same fashion.

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[14] Mutation Theory, 1909, 1.

A description of some of the mutations of Nicolaierillus strains will be presented in a future paper. They are frequent enough to explain the wide range of colony form found in the genus. The bearing of this phenomenon on the taxonomy of the genus is probably nil. Other workers in this laboratory that are using the same strains may be able to confirm the results of Tulloch or to assign certain colony types to various other groups of their own; in other words, to attach some in the vegetative form is not taken advantage of. In many mediums chemical significance to the change in colony form. It is premature even to select a "type species" for the genus. Till further study is made. type 1 of Tulloch, identifiable only by an agglutinating serum, may be taken for a so-called "type species" with the name "N. tetani." A chemical or morphologic character rather than a serologic one is of course preferable for the subdivision of a group.

THE ISOLATION OF NICOLAIERILLUS STRAINS

Many complaints have been made of the difficulty encountered in isolating these organisms. This is due to several factors. In mixed cultures the organisms are recognized only when they form spores—a comparatively late period—and their early active multiplication phase they are somewhat overgrown by other proteolytic organisms. It is extremely difficult to get tetanus bacilli to grow on the surface of an agar plate that is free from blood. In deep agar the colonies of different strains are so unlike that no widely representative "type" can be recognized. The strains that form minute colonies are perforce incapable of easy isolation from gross mixtures. Of interest is the fact that Righi accepted as typical of tentaus bacilli colonies with a dense center and loose radiations, while a collection of cultures sent me by Dr. Duval of New Orleans that were probably isolated by one worker, all made large opaque woolly colonies. Tulloch devised a selective medium for B. tetani by means of which Robertson was able to isolate the organism from wound cultures by the Barber technic. McCoy and Bengtson [15] isolated a series of strains which they were so kind as to furnish this laboratory. Their isolations were made as follows: Fermentation tubes were filled with freshly boiled veal infusion, inoculated with suspected material, and incubated at 37 C. until spherical spores were observable in the cultures. Such a fermentation tube was then heated at 70 degrees for 30 minutes and material from it was inoculated into deep agar shakes of veal infusion agar, and the colonies were fished after 3 days' incubation. I have not tried this method in its details, but give it here because it is so simple. When tetanus strains have become contaminated in the laboratory I have had little or no difficulty in isolating the types of active growth by deep colony procedure from liver peptone agar. For some time I was baffled by mutating colonies, and I believe that many supposed contaminations by other anaerobes were cases of mutation. Meat medium should be avoided as a substratum on which to propagate contaminated tetanus strains as it encourages too many other forms. When dealing with badly overgrown cultures or with material from wounds, feces or soil, such a medium as Tulloch's is to be recommended, followed by a deep-colony procedure. The only element that will then make the isolation of the bacilli difficult is the worker's ignorance of the colony form of the particular strain that he desires to isolate. He must, in most cases, fish more colonies than he would were he dealing with a genus of uniform colony formation. The illustrations will help somewhat in recognizing various sorts of tetanus colonies. In case a strain is present that forms minute colonies, one may try other agar substrata, or Barber's technic,[16] or one may use many dilution tubes and incubate for 3 or 4 days until the minute colonies in one or other of the tubes are large enough to be fished readily. Tetanus colonies grow with fair speed at room temperature, and in some cases it may be found advantageous to incubate at a low temperature. For fishing crowded colonies the binocular is of great assistance.

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[15] Am. J. Publ. Health, 1919, 9, p. 427.

Robertson (per. com.) notes that tetanus cultures become more readily contaminated than do those of other anaerobes. I formerly noted the same phenomenon, and believe it to be due to the fact that she and I usually reincubated tetanus cultures in anaerobe jars to observe spore formation. Long incubation in anaerobe jars frequently causes contamination, as does also the exhaustion of meat tubes containing gas, in which the medium is allowed to boil up onto the cotton plugs. I find that incubation of meat medium cultures of tetanus bacilli in air with or without vaseline allows characteristic development of the cultures.

[16] Phil. J. Sc., 1914, 9, p. 307.

THE BEHAVIOR OF NICOLAIERILLUS STRAINS IN MEAT MEDIUM

Statements concerning the proteolytic activity of the tetanus bacilli vary considerably. There is almost entire unanimity that gelatine is liquefied. Von Hibler[10] and Zeissler,[17] on the one hand, ascribe to tetanus organisms an intense proteolytic activity. Von Hibler worked with badly mixed cultures. He states, however, (p. 95) that many strains of tetanus bacilli do not blacken brain deeply. Zeissler goes so far as to state that brain is blackened intensely and that the odor varies between a pungent odor, and a very bad one, and that milk is peptonized in from 4 to 14 days. His photograph apparently shows a culture contaminated by oval-end-sporulating organisms. On the other hand, the Medical Research Committee[12] state: "Meat medium: Pink color or no change in the medium according to the samples of meat; softening of the consistency of the meat. The odor is characteristic but not putrefactive. Milk: Poor growth; no change in medium. Coagulated serum: Little or no liquefaction." Adamson[18] found the organism mildly proteolytic.

My experience would lead me to emphasize the proteolytic action of the organism a little more than do the members of the Committee. Perhaps they worked with a peptone-free meat medium that did not afford so much nutriment for the ogranism as that employed in this laboratory. But such descriptions as that of Zeissler cannot be construed as applying to pure cultures of tetanus bacilli, for these are among the less active proteolytic anaerobes. The Committee is certainly correct in stating that the color change in meat medium varies with the batch of medium. A series of pure tetanus strains inoculated and incubated uniformly on one batch of medium behave with striking similarity—those that form minute colonies on agar cause changes in the medium with less rapidity than do those that form large colonies. Meat medium is made in this laboratory of one part of coarsely ground beef heart with two parts of distilled water. Five hundred c c of Martin's peptic-digest broth is added to 3 liters of meat medium. The reaction is P_H 7.2. For a long time it was thought that the behavior of tetanus bacilli on this medium was exceedingly uniform and characteristic. Later other assistants made the medium and separate batches were found to give very different results. All batches with an initial reaction in the neighborhood of P_H 7.2 gave evidences of moderate proteolysis inside of a few days and showed slight softening

[17] Ztschr. f. Infektionskrankh. d. Haustiere, 1920, 21, p. 1.

[18] J. Path. & Bacteriol., 1920, 23, p. 241.

of the medium, and never, even on long incubation enough softening to cause more than a slight settling of the meat particles. In odor they were much the same, and the morphologic aspect of the bacilli was much the same. But the color changes were different in the various batches of medium.

The most usual type of color change noted was an initial yellowing of the meat, followed shortly by the appearance of a pinkish shade that changes later to a light terra cotta, rarely to a dark terra cotta. A black pigment is usually noted suffusing the particles nearest the surface and a few of the morsels lower down. If the particles be finely ground, the top layer later bleaches in a few days to pale yellow. If the incubation is in hydrogen a diaphanous black ring appears at the surface of the liquid, but disappears on exposure to the air. Some batches of medium show the initial yellowish stage protracted, and the pink color does not become intense. The black pigment is then very tardy in making its appearance, or it may be absent. In other batches neither pink nor yellow appear. The meat becomes gray and apparently allows good growth of the bacilli. Black pigment may be present. Another batch that apparently afforded excellent growth early gave a mauve color suffused with black pigment. A lot of meat medium that titrated P_H 6.6 sometimes failed to give any growth with tetanus bacilli, but when it did the organisms did not change its appearance. A collection of meat medium tetanus cultures that was three months old showed considerable variation in reddening and blackening. This is consistent with the ready mutation observed with some strains on protein medium. Cultures a year old that have been left at room temperature in air are not uncharacteristic in appearance. If the column of meat is high, it will be noted that all sharp edges of the particles of meat have disappeared, but that the particles have not settled greatly in the tubes. Their basic color may still be red, but this color is almost coated by a thin black film. The top layer of meat a quarter of an inch thick is almost white, the boundary between this layer and the blackened meat below being sharp if the meat has been finely ground.

Gas production is abundant early and becomes less after a few days.

Most authors agree that the odor of tetanus cultures is characteristic and not like that of other proteolytic anaerobes. A young meat culture is faintly and not unpleasantly proteolytic and no butyric acid or sourish smell is noticeable. After 10 days the odor at the mouth of the tube is of hydrogen sulphide only, that of meat smeared on a slide is pungent and different from that of most proteolytic anaerobes. Most people would term it unpleasant. At 3 months the odor is rank, pungent and almost overpowering.

Bacilli are moderately abundant in 24-hour meat medium cultures whether incubated in air, under vaseline or in hydrogen. They are most abundant in 48-hour cultures and do not drop off in numbers abruptly after this time as do so many anaerobes. This is the most marked distinction to be noted between the tetanus bacilli and the nonproteolytic Macintoshillus bacilli (Bacillus tetanomorphus [10, 11]) when stained cultures are observed. Vegetative rods are quite characteristic in their morphology. Their thickness is very uniform, rarely far from 0.8 : 1.0 micron. The length may vary from 1 micron to very long filamentous rods. The usual length is from 2-8 microns and filaments and long rods and attached bacilli are rare. The rods are nearly always straight and the ends neatly rounded. They are uniformly and consistently gram-negative when treated with a weak solution of methyl violet, and are gram-positive after treatment with a strong violet stain, such as Sterling's. The gram-negative bacilli are remarkably clear in their staining with the carbol-fuchsin counterstain, and there are no pale forms and no bacilli showing irregularities of protoplasm in cultures under 5 days of age. The morphology of the nonsporulating tetanus bacillus, at first insignificant and hard to distinguish from that of other organisms, becomes to the experienced eye characteristic and definite, and contaminations of tetanus cultures are usually readily noticed. The anaerobic tetanomorphous bacillus of McIntosh and doubtless some other organisms are, however, not definitely to be distinguished by their morphology alone.

Spore formation may commence within forty-eight hours but usually begins on the third or fourth day and is active for a long period; occasionally it fails to appear. Spores vary considerably as to the time of their formation and as to their abundance in the different strains. Spores are invariably spherical and orgonts [19] (p. 4 or 389) show an oval end only in the very early stages. Swelling of the rods takes place apparently only in the portion where spore formation occurs. Spores remain in the rod for a long period. Orgonts are not abundant, sporulating rods are frequent, and only after 10 days or so does one note numerous free spores. The majority of spores are, in most strains, strictly terminal, but occasionally a strain is found that repeatedly forms some spores centrally. A tip of protein from the rod is occasionally noted on the distal end of the spore, giving the rod an appearance superficially like that of a sporulating sporogenes or botulinus organism. But the wall of the spherical tetanus spore is thinner than in those species, and the tip of protein more rounded, the sides of the bacillus are more nearly parallel. I have rarely observed two spores in the same rod. The bacterial wall about the spore is thin, and the spore wall is thin; refractility is low. Sometimes the spores themselves retain the methyl violet of the Gram stain to some extent, remaining transparent the while. The spores of Macintoshillus do the same; both types occasionally show young spores thickly covered by the mother cell, and these may stain gram-negatively like the mother cell or they may retain the stain. Macintoshillus bacilli form more spores during the first two days than Nicolaierillus bacilli. Colonies of the former organisms are pictured in this article.

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[19] Heller, H. H.: J. Infect. Dis., 1920, 27, p. 385.

NICOLAIERILLUS STRAINS ON OTHER PROTEIN MEDIUMS

The behavior of tetanus strains on protein mediums other than meat has been so well treated by Adamson[18] that extensive experiments have not been tried. The following notes were made on the behavior of five pure strains that were incubated in hydrogen at 37 C.

Indol broth gave good growth and after five days of incubation a negative test for indol.

The bacilli grew well on brain medium, producing at first a little gas and abundant rods. After four weeks the medium was not blackened, nor did it show any sign of a greenish or grayish tinge. The lower portion was pink as it had been before inoculation. The odor was faint and ester like, in no way repellent and putrefactive.

The organisms apparently refused to grow on alkaline egg.

Deep coagulated beef serum furnished active growth, and at first gas. Spores were formed early and abundantly. The medium soon showed a gray-greenish color, and at the end of a month was suffused with a slightly greenish leaden tinge and was somewhat softened but not liquefied. The odor was faintly proteolytic.

Although there was multiplication on deep Loeffler's serum, the bacilli did not produce a greenish color. The odor was musty.

In deep Dorset's egg gas was formed early and a slight softening of the medium took place. Later lead-colored spots appeared with a general pale suffusion by the lead color. The odor was faintly of hydrogen sulphide.

Brom-cresol milk was clotted by a rennet like enzyme of slow action. The clot appeared during the first week, and at the end of four weeks most of the casein had settled to a somewhat rubber-like clot at the bottom of the tubes. This clot was compact and could be torn in pieces that disintegrated in invisible particles. The mass behaved much like a compactly settled lump of white lead in paint. The supernatant liquid was not clear, but was clearer than normal milk, and alkaline. Peptonization, if present, was certainly not visible to the eye. The odor was musty.

SUMMARY

Certain differences may be found between Nicolaierillus strains on careful search. It is premature to apply these differences to specific differentiation.

Tetanus strains show remarkable powers of mutation on complex protein substrata. These powers are easily observed in colony formation, but do not affect grossly the picture of proteolytic action on meat medium.

The morphologic and staining reactions of the bacilli are very characteristic and permit the accustomed worker to recognize them readily and to spot contaminations with ease. The behavior of pure cultures on meat medium is fairly characteristic and is different from that of most common contaminating anaerobes. Meat medium is probably the best protein medium for the differentiation of tetanus strains from other anaerobic organisms. All strains observed were noticeably proteolytic and none were intensely so.

Such strains as form large colonies are easily isolated by deep colony procedure.

APPENDIX. LIST OF TETANUS STRAINS STUDIED

From the Hygienic Laboratory, Washington, D. C.
Agar stabs. May, 1917.
THL = "Tetanus No. 1 Hyg. Lab."

November, 1918.
TA = "Tetanus Ag. Dept."
TB = "Tetanus 2 NY Dept. June, 1919."
TC = "Tetanus Courtplaster."
TD = "Tetanus P. D. Co."
TE = "Tetanus 089."
TG = "Tetanus from gelatine."
TM = "Tetanus Mulford."
TN = "Tetanus N. Y. Board of Health."
TO = "Tetanus OT 026580."
TP = "Tetanus Smith's from pease."
TR = "Tetanus OTK 02."

TT = "Tetanus Tulloch type I."
TU = "Tetanus U. Chicago."

June, 1919.
TVP = "Tetanus from vaccine virus point."

From Miss Muriel Robertson, Lister Institute, London.
TMcC = "Tetanus of MacConkey" London, Nov., 1916. Meat culture.

November, 1918.
TUSA = "B. tetani USA II" Tulloch's type I. Egg broth.
T220 = "B. tetani R 220 L" Tulloch's type III. Meat culture.
T67 = "B. tetani T 67 V" Tulloch's type II. Egg broth.

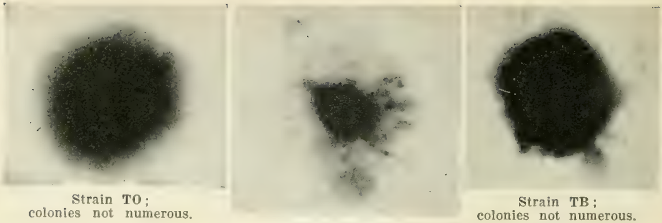
From Dr. K. F. Meyer, San Francisco.
VT = "Tetanus from vaccination case." A small boy who had been vaccinated played in a stable and developed tetanus and died. Two types were cultivated from the culture made from the vaccine pustule.

From Parke, Davis and Company, Detroit.
T 087 = "Tetanus 087" Agar stab.

From the Gilliland Laboratories.
TL = "B. tetani" Agar stab.

From Dr. Duval, New Orleans. Agar stabs.
T 2 A = "B. tetani 204A." Isolated from wound.
T 2 B = "B. tetani 204B." Isolated from soil.
T 2 C = "B. tetani 204C." Isolated from wound.
T 2 E = "B. tetani 204E." Isolated at necropsy.
T 2 F = "B. tetani 204F." Isolated from wound.

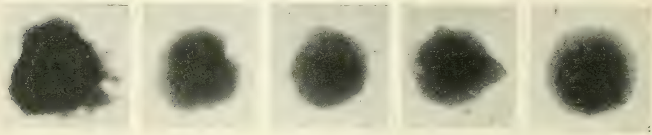
From the Cutter Laboratory, Berkeley.
T 4 = "B. tetani 4." Agar stab.



Strain TO;
colonies not numerous.

Strain TMcC;
colonies few.

Strain TB;
colonies not numerous.



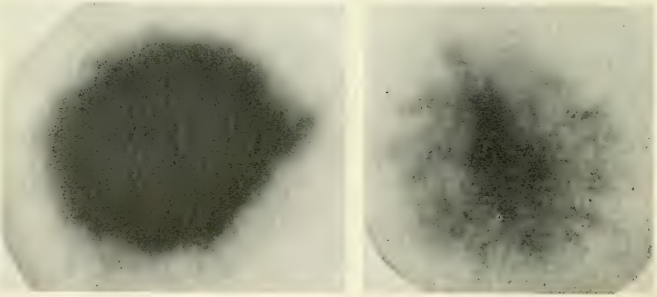
Strain TC;
colonies not numerous.

Strain VTS.

Strain TA.

Strain TG.
5 days, × 15.

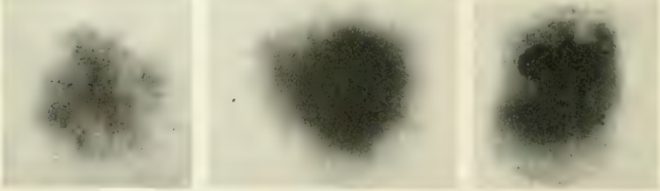
Colonies few.



Strain T_2F.

Colonies not numerous.

Strain T_4.

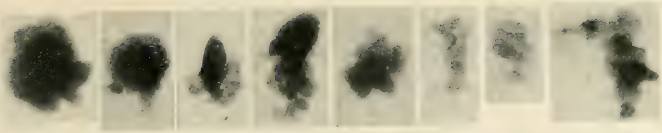


Strain TN;
colonies not numerous.

Strain T_2A.

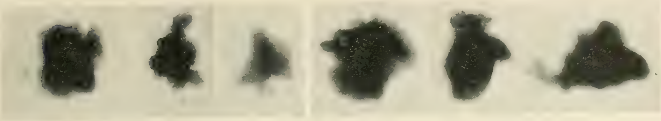
Strain TG; 5 days, × 15.
Colonies few.

Fig. 1.—Various types of Nicolaierillus (Tetanus) colonies. Unless otherwise indicated, these photographs represent colonies that were incubated for 24 hours at 37 C. in deep liver peptone agar and were enlarged 50 diameters.



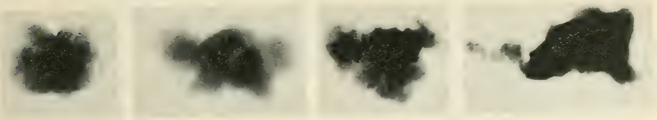
Strain TE; colonies few.

Colonies moderately abundant.



Strain TU; colonies not numerous.

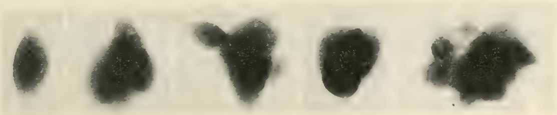
Colonies few.



Strain TE; 48 hours' incubation; colonies moderately abundant.

Strain TU

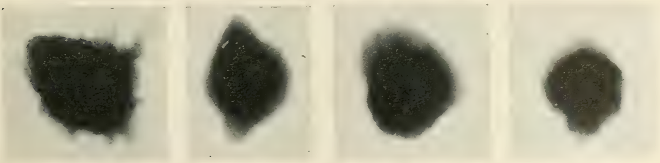
Colonies moderately abundant. Strain TD.



Four days' incubation; colonies few.

NICOLAIERILLUS (TETANUS) COLONIES

Macintoshillus tetanomorphus, type strain, PT; colonies abundant.



Strain 297.

Strain 221.

Colonies moderately abundant.

Fig 2.—Macintoshillus (B. tetanomorphus) colonies.

CLASSIFICATION OF THE ANAEROBIC BACTERIA

HILDA HEMPL HELLER

Reprinted for private circulation from
The Botanical Gazette, Vol. LXXIII, No. 1, January 1922

CLASSIFICATION OF THE ANAEROBIC BACTERIA[1]

HILDA HEMPL HELLER

The classification of living forms should depend on an understanding of the laws of heredity as demonstrated in those forms. Preliminary classifications are made by applying the machinery of arrangement that has been worked out for other groups that are well understood, to groups of whose biological processes we know little. Preliminary classifications are necessary and are as desirable as are catalogues, and should be made to correspond to the known life processes of the organisms as nearly as possible, but they should only be offered tentatively.

The study of the biology of the anaerobic bacilli is in its early morning twilight. Today the scientific world holds two widely opposite opinions in regard to the classification of these organisms. The view held in Western Europe and in America is that the anaerobic species are many, distinguishable, and not highly variable; that held by many workers in Central Europe is that the species are practically indistinguishable, are highly variable, and may be changed one into another. It is impossible to bring these two points of view into alignment. They are not to be attributed to diverse interpretation of differences that shade into one another. They are themselves the outcome of an evolutionary process, depending on a mutation in thought, followed by the throwing up of a geographical barrier in 1914 that isolated the mutant thought and permitted it to overgrow its ancestral types of reasoning in a peculiarly favorable soil. The crux of the matter lies in the purity of the cultures studied by the classifier. It is notorious that the casual worker with anaerobic organisms knows neither how to purify them, nor how to tell when they are pure or impure. The anaerobes are not difficult to isolate when one knows how, but usually workers do not know how. Anaerobes have occasionally been isolated in pure culture since the early days of bacteriology.

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[1] From the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco.

A number of descriptions of pure cultures exist, but it was the exceptionally studious worker who was responsible for such descriptions, and casual workers were apparently in the majority.

In 1901 GRASSBERGER and SCHATTENFROH (18, 19) propounded their theory of the transmutability of anaerobic species. In the period following they corroborated and extended their findings, and their work was pushed with so many publications (16, 17, 20–23) and with so much assertion that by 1914 it was seriously quoted in at least one well known German textbook (39), and the doctrine was thoroughly distributed throughout Central Europe. Under pressure of war, work is not carefully done. The casual workers found it necessary to make many anaerobic diagnoses from gas gangrene cases, which they made rapidly, and then turned to their new found collections to classify them. Many of these war workers corroborated the general findings of GRASSBERGER and SCHATTENFROH, namely, that the characters of anaerobes are highly variable, and that species among these organisms are not to be seriously studied.

CONRADI and BIELING (4, 5) were the most extreme in their contentions, claiming that one labile species was responsible for all gas gangrene cases. They described two cycles, one developing on carbohydrate media, the other on protein media, and claimed that immune sera identified all the strains in each cycle, but that when a strain was changed over to the other cycle culturally, it was also changed immunologically to that cycle. Such contentions struck so forcibly against all conceptions of immunity that they did not long go unchallenged. A number of workers (12, 38) corroborated the transmutability findings but not the immunological ones. In fact KLOSE (31, 32), working with highly variable impure cultures, used toxin formation to distinguish his strains. Some, such as ASCHOFF (1), who worked with slightly impure cultures, remained on the fence in regard to the transformations. Most assertive in their contentions that anaerobic organisms are highly variable in their reactions and are transmutable were the school of KOLLE (34–36), especially SCHLOSSBERGER (43), who suggested that the anaerobes may represent a single species. These workers sent cultures to VON WASSERMANN (49), who declared them indistinguishable. He refused to submit anaerobic cultures to such drastic treatment as transatlantic shipment because of their fragility (48), and yet an anaerobic organism taken in muscle from a whale by NIELSEN (40) in 1888 responded satisfactorily to routine anaerobic technique in this laboratory in 1918, killing its guinea pig promptly and resembling exactly its original description! In the veterinary field VAN HEELSBERGEN (24) recently aligned himself with the German workers in human pathology.

The alteration of scientific attitude brought about by the adherence to such a theory as this is most interesting. Things become so simple under such an explanation, many technical difficulties are eliminated, immunization of animals for therapeutic purposes is made easy, and the scientific world, from the point of view of these workers, is so much the better off. For, if anaerobes may be changed one into another, why bother about isolating them? They will not stay pure. Anaerobic cultures from Central Europe that have found their way to this country are seldom pure, and frequently do not contain the type of organism for which they were named. If they contain a pathogen, two or three types may be isolated from the cultures, and these types behave consistently and do not do the queer things they were supposed to do by their first students. Central European anaerobic studies are struggling in the dark, the days of NÄGELI and BILLROTH have returned, the land of KOCH (33), of GHON and SACHS (15), and of VON HIBLER (30) has shifted from the careful work of older days, GHON himself (14) is converted to the new theories, and but a few constructive workers with abundant material have come out openly to combat them. Chief among these is ZEISSLER (51), a pupil of and coworker with FRAENKEL (6–11), who has always maintained that gas gangrene is due to various distinguishable anaerobic organisms. PFEIFFER and BESSAU (41) and GAEHTGENS (13) clearly distinguish various types. Early in the period of the war ZACHERL (50) and KÖVES (37) gave good descriptions of pure cultures of the vibrion septique type of organism.

As is to be expected, museum cultures from Central Europe are more badly mixed than any others. A collection of ten strains of anaerobes from KRAL'S in Vienna apparently did not contain a single species for which the cultures were named. Unfortunately the anaerobe strains of some of our own institutions are but little better.

It would be difficult for the systematist employed in the study of higher plants whose major characters are well understood, whose mutations are today being scientifically studied, whose formal structure of classification was laid down many years ago and has been systematically developed, to imagine the complexity of the problem confronting one desirous of bringing order into the chaos represented not only by this war literature, but also by thirty years of anaerobic literature written before it. The time is more than ripe for some organization to enable new students to set to work with some clearness and assurance, an organization with a synopsis or index to the enormous literature that they must consult. This should give them an idea of the multiplicity of the species they will encounter, and should consider the biological factors relating to morphology, chemical behavior, and mutation as they are understood today.

Several workers have stated that anaerobic bacilli do not mutate. This is their natural reaction in denying the existence of the type of mutation that was described by the workers with impure cultures. To state that a living strain does not mutate would be to claim that it lacks one of the best recognized attributes of living matter. Obviously, it is necessary to determine where the mutations of bacteria lie, and what range of possible change they cover, before one can tell what characters are stable enough to be used for systematic purposes. The enzymes of the anaerobic bacilli are among the most highly active chemical agents known. Some of the anaerobes will be found among the most active splitters of carbohydrates, others have almost unbelievable proteolytic powers. It is to be expected that mutations will frequently be encountered in highly specialized organisms of this sort, and that these mutations will be chemical in their nature. When a mutation occurs that enables the organism to render assimilable a substance that its parent was unable to utilize, the mutant is readily detected because of the larger colony that it produces. Likewise a bacillus that loses a metabolic power forms smaller colonies than its parents. Data pertaining to such mutations may be recorded photographically (28), and the possibilities thus afforded for the study of bacterial mutations in certain groups, notably in that of the tetanus bacillus (29), are unlimited.

The Society of American Bacteriologists (2) proposes the use of the botanical rules adopted at the Vienna Congress (44) in 1908 for the purposes of bacterial classification. In many ways the scheme formulated during a century and a half by the botanists is excellent for the purpose, although in some ways we are not ready for it. It is composed of stems and twigs and branches. When we pick up a bacterial group, we do not know whether to call it a stem or a twig or a branch, for the leaves have mostly grown on trunks. The tendency has been to work downward, to call a superficially recognized group a species and subdivide it into types, and to number the types. Why not work upward, call the numbered types species, and have more room for classification?

Bacteriologists, trained in pathological laboratories, have perhaps laid too little emphasis on the necessity of observing the laws of heredity in making classifications. It seems as though an application of these laws, with the same scale of nomenclature used by the classifiers of higher plants, might well be applied to the systematic arrangement of bacteria. Thus a tetanus strain of a pure biotype may give rise to many biotypes, as shown by colony formation. These derivative types are all typical tetanus bacilli. They represent elementary species, and are too many to catalogue, being of interest only to the student of heredity. They are no more deserving of specific names than are the commonly observed small mutations of higher plants and animals, and if named would require a trinomial nomenclature. There are some definite protein substances, however, differing radically in various tetanus bacilli, that probably are not subject to active mutation and are demonstrable by an immune reaction, agglutination. Four groups of tetanus bacilli have thus been distinguished by TULLOCH (**45, 46**), and four groups of vibrion septique bacilli by ROBERTSON (**42**). In the colon-typhoid group this reaction has long been considered specific, why not in these? To be sure, the details of the reaction in these groups require more study, and other and better ways may be found to divide them. The following general rules will probably be found convenient for classifying bacteria.

BIOTYPE.—Strains that differ from each other in characters that are readily subject to mutation, and that breed true, may be termed biotypes. The word subspecies has so long been used in the higher groups with a geographical connotation that it will not be well to use it for subdivisions of bacterial species. The term type may then well be left as an independent unit of our vocabularies for non-specific use.

SPECIES.—Strains that behave alike in those characters that within their genus have not been found to mutate readily, may be grouped as species. The occasional derivation of one species from another is no more to be considered impossible than it is in higher groups. Bacteriologists have too long considered the species conception in higher groups as one of fixed immutable forms, which it is not. The recombination effects noted by DE VRIES (47), in which he showed the independent origin of three well defined types (*Oenothera nanella*, *O. eliptica*, and *O. lata*) from two others of quite widely divergent character (*O. Lamarckiana* and *O. laevifolia*) apparently cannot occur among bacteria. These recombination effects allowed the sudden appearance of groups of mutations that had occurred previously. Among bacteria, however, because of the rapidity with which vast numbers may be bred and the energy with which selection acts, several characters may be changed nearly simultaneously, and similar effects to those noted by DE VRIES may occasionally be observed, namely, the appearance of a number of new characters within a short space of time. On analogy, it would be perfectly reasonable to describe the strains that result from various changes as separate species. It is quibbling to define the word "species" so closely that no elasticity should be allowed in its application. Our knowledge is too meager and the possibilities too great to restrict closely the meanings of taxonomic words. Changes that may be considered specific may be discovered or perhaps even brought about by treatment of bacteria that is more radical than anything tried with plants, and there is no reason why a bacteriologist who finds a new type that has originated in his own hands should not dignify it by the name of species, if he can show that such a change affects several characters.

GENUS.—Organisms that show the same general reactions on ordinary media and have the same general morphological habit may be grouped in genera. Such a scheme will compel the classification of most of the old and well recognized anaerobic species as genera, although it will unite some, such as NOVY'S bacillus and *B. oedematiens*, and vibrion septique and the whale septicaemia bacillus into genera. It gives to the words species and genus approximately the same rank, in relation to mutational possibilities, as they possess among the higher forms. A vibrion septique strain can no more mutate to a sporogenes habit than can a pine tree mutate to an oak, but it can mutate in small detailed characters that may be of interest, and there are vibrion septique strains that differ in more fixed characters and that should be given specific differentiation.

A detailed plan (27) following these lines has already been presented. It is intended as a preliminary classification. Twenty-seven anaerobic genera have been defined, many more will have to be admitted in time, especially in the proteolytic group, and when large collections have been studied many emendations will have to be made. An organization (26) of these genera has been prepared, employing chemical criteria only; morphological criteria are found entirely inadequate for purposes of classification in this group. It has been thought premature to group the genera into tribes. Two subfamilies include all the anaerobic rods described in the genera.

Subfamily I. Clostridioideae.— Clostridiaceae that on meat medium produce after twenty days' incubation under oil at 37° a reaction of P_H 7.0 or a more acid reaction, the culture having been boiled after incubation. Type genus *Rivoltillus*, the vibrion septique type as described by HELLER (25).

Subfamily II. Putrificoideae. Clostridiaceae that on meat medium produce after twenty days' incubation at 37° under oil a reaction of P_H 7.1 or a more alkaline reaction, the culture having been boiled after incubation. Type genus *Metchnikovillus*, the sporogenes type as defined in the description of *Bacillus sporogenes*, described by the Medical Research Committee (3) under the name of Metchnikoff's type A. These subfamilies are united into the following family:

Clostridiaceae.—Eubacteriales that are rodlike, not spiral, that will not grow within 7 mm. of the surface of a shaft of clear tissue-free agar medium contained in a tube 12 mm. or more in diameter, incubated in air, in which they are able to grow in the depths. They may or may not possess peritrichial flagella; they may or may not form endospores. Most members of the group are characterized by their energetic catalytic action on proteins or on carbohydrates or on both of these types of substances.

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A DETAILED BACTERIOLOGIC STUDY OF A CASE OF GAS GANGRENE FOLLOWING A COMPOUND FRACTURE

STUDIES ON PATHOGENIC ANAEROBES III *

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CLINICAL OBSERVATIONS

A summary of prewar cases of gas gangrene has shown that this type of infection occurs far more often with compound fractures than with any other type or condition of wound, and that the occurrence of gas gangrene in civil life is extremely uncommon, only 175 cases having been reported up to 1915. Data on this subject since the war show that the percentage of gas gangrene cases in war wounds was moderate, ranging in different localities from 1 to 3.5 per cent.

In the 175 cases collected from the literature up to 1915 by Simonds,[1] the relative number of several types of wounds was as follows: compound fractures, 61; lacerated wounds, 28; operation wounds, 11; gunshot wounds, 10, and hypodermic needle puncture, 9. Thus nearly as many cases of gas gangrene developed in hypodermic needle punctures and slightly more in operation wounds than in gunshot wounds. Gas gangrene was twice as frequent in lacerated wounds and six times more frequent in compound fracture cases than in gunshot wounds. This refutes the impression gained from war reports that gas gangrene was peculiarly incidental to gunshot wounds. Furthermore, the relatively high frequency of gas gangrene in compound fracture wounds as compared to some other types of wounds, which are equally liable to contamination by foreign substances, argues for a different accessory agent to the growth of gas gangrene organisms than is supplied by the nidus theory.

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1. Simonds, J. P.: Studies in B. Welchii with Special Reference to Classification and Its Relation to Diarrhoea, Rockefeller Institute for Medical Research, Monograph 5, 1915.

In the experiments of Bullock and Cramer,[2] particles of wood, cloth, paper and other foreign substances, after being soaked in a suspension of *B. welchii,* were introduced beneath the skin of mice with negative results. *B. welchii* was also introduced into hematomas with negative results. Bullock and Cramer found, however, that the presence of certain calcium salts aids in the production of gas gangrene. Doses of 2½ mg. of calcium chlorid injected with a suspension of *B. welchii* or of *Vibrion septique* never failed to produce gas gangrene in the experimental animals. Calcium nitrate and calcium acetate have the same effect. The conclusion of Bullock and Cramer was that calcium salts produce at the site of injection a local change in the tissues which lessens their defensive mechanism. Therefore, does it not seem probable that calcium salts, liberated by the disintegration of devitalized bone substance in compound fractures, may supply, to a large extent, the factor that lessens the resistance of the tissues to gas gangrene anaerobes?

Whether a fracture is compounded from within outward by a bone fragment or from without inward by a foreign body, there is in either case ample opportunity for bacterial invasion. But in compound fractures, not only the severe traumatization of the bone substance itself, but the usual traumatizing effect of the bone fragments on soft tissue cause far greater devitalization than would result in a corresponding injury to soft tissue without fracture. The increased devitalization burdens both the injured and adjacent tissues with a greater metabolism complex and no doubt a lowered cell resistance. This increased devitalization, which includes both bone and soft tissue, is probably an important accessory cause for the increased susceptibility of compound fracture over other wounds for the development of gas gangrene.

REPORT OF CASE

History.—M. R., aged 8 years, schoolgirl, American, entered the surgical clinic of the University of California Medical School, Oct. 2, 1919. On the evening of October 1, the patient fell from a bench, 4 feet above the ground, injuring her arm. The ground was covered with dry grass and showed nothing to suggest any particular contamination. The child's home was close by, and next to a barnyard. There were several goats in the vicinity.

Immediately after the accident, the child, with the injured arm wrapped in a towel, was taken to an emergency hospital, where at 8 p. m., the fracture was reduced and the arm was splinted.

Examination.—When the patient entered the surgical clinic the next morning, the entire forearm was moderately swollen, but not more so than would be expected from a fracture of both the ulna and the radius. Over the middle third of the ulna on the volar surface was an open wound, 1.5 cm. in length.

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[2] Bullock, W. E., and Cramer, W.: On a New Factor in the Mechanism of Bacterial Infection, Proc. Royal Soc., Series B **90**:513, 1919.

Inquiry elicited the fact that before reduction at the emergency hospital, the lower fragment of the ulna was protruding through the wound. At the time of examination at the surgical clinic, fourteen hours after injury, no symptoms nor signs of gas gangrene were noted. But the following morning, October 3, when the child returned to the clinic, the forearm was gangrenous, with circulation stopped just below the elbow. The entire arm was greatly distended, and edema had extended into the shoulder muscles. From gangrenous blebs on the wrist, when punctured, oozed a bloody serum and bubbles of gas. Gas bubbles also issued from the wound.

Operation and Result.—The arm was amputated, guillotine method, just below the level of the insertion of the deltoid muscle. The amputation was well above the lowest line of the circulation. Smears made at this time from the wound in the forearm showed the presence of *B. welchii.* No tetanus organisms were found; but 1,500 units of tetanus antitoxin was administered subcutaneously.

On the morning following the operation, 20 c.c. of *B. welchii* antitoxin, which was the only available antiserum related to gas gangrene, was given intravenously. We were able, fortunately, to obtain *B. welchii* antitoxin through the courtesy of Dr. K. F. Meyer of the Hooper Foundation. On the second day following the operation, 15 c.c. of this antitoxin was given subcutaneously, about 50 antitoxin units in all. The child was apparently very toxic. A marked tachycardia developed, together with restlessness, delirium, stupor and cyanosis. The wound was treated with surgical solution of chlorinated soda (Carrel-Dakin solution). It was dark in color, with patches of crusts covering sloughs. The patient remained in restless delirium until the eighth day after operation; the pulse rate increased to 160, and the temperature to 39.2 C., rectally. There was a blowing systolic murmur heard over the precordium; the liver edge was 3 cm. below the costal margin; but the splenic margin was not palpable. There were also, on the eighth day, definite signs of serum sickness with edema of the eyelids and lips and herpes on the lips. There was also vomiting and diarrhea. The wound had not begun to granulate but was still the color of old meat. The Carrel-Dakin solution was discontinued, and a mild solution consisting of salt solution and hydrogen peroxid was substituted.

On the following day, the ninth day after operation, the pulse rate began to drop; the child became rational; the sloughs began to disappear, and the child advanced steadily to complete recovery. She was dismissed from the hospital on the twentieth day after operation.

PATHOLOGIC ANATOMIC NOTES

The examination of the arm amputated was undertaken three hours after the operation. The odor of the arm was strong, rank and penetrating, but not putrid. It resembled that of butyric acid; but there was also a rank, peculiar odor similar to that of blackleg muscle in cattle. The arm was rigid as it had been before amputation. The tissues were about as resistant to pressure as a very hard rubber ball, the moist skin of the forearm showing considerable stretching and tension. On the wrist and on the palm of the hand were several long bullae filled with a serous colorless liquid. The hand and fingers were very edematous and had a white transparent appearance. Crepitus was present in the hand, wrist and forearm. On the flexor surface, midway between the wrist and elbow, was a small wound about $\frac{3}{4}$ by $\frac{1}{4}$ inch in area. This had probably been caused by perforation by the fractured ulna. The forearm showed blue marks and abundant indications of contusions. There was no bronzing of the skin unless a certain dark discoloration of that on the wrist may be so designated. The elbow was greatly swollen as was the upper arm, but above the elbow crepitus was absent.

The cut end of the arm showed a bubbling of gas at the site of the principal blood vessels. A large amount of blood escaped from the arm before it was examined, probably on account of the gas pressure.

An incision made along the flexor surface revealed a varying amount of gas in the different portions of the arm. Most striking was a gradual spreading of the skin of the wrist, till it lay open for a space of more than an inch. Gas was released from solution where it had been confined by the pressure exerted by the integument, and large bubbles appeared in the connective tissue and among the muscles, distending the tissue greatly. The muscles in this region were slightly dry and somewhat hemorrhagic. They were not brick colored or yellowish nor had they in any great measure lost their elasticity. Section of the palm allowed considerable bubbling to take place; but it was apparent that most of the swelling of the hand was due to a white edema that retained its gelatinous consistency on section. The muscles of this region were normal or slightly pale in color; all the tissues were moist and elastic, and there was no turbid exudate except a little fluid that contained a few red cells. The large muscles of the upper portion of the forearm were moist, edematous and contained a considerable amount of gas. This bubbled forth for some time after section without the application of any pressure. Its odor was rather rank and sweetish, the butyric acid element not being marked, and a true proteolytic odor not being noticeable. All the muscles were hemorrhagic, most of them uniformly so. The fracture of the ulna had occurred in the middle of the shaft; that of the radius was comminuted and in the upper third of the shaft. Examination of the broken ends showed that the arm had been shortened a quarter of an inch, perhaps partly by the pressure of the gas. On the muscle next to the fractured end of the ulna was a yellowish discoloration thought to be due to bacterial action. Bacteria were, however, not found to be especially abundant in this region. The muscles of the forearm surrounding the fracture were somewhat darker, and the region immediately about the fracture of the radius was marked by the presence of much gas and areas of black hemorrhagic infiltration of the muscles. Extending toward the elbow, several of the muscles showed irregular, small and large black hemorrhages. On the flexor surface of the elbow was a small lacerated blood vessel

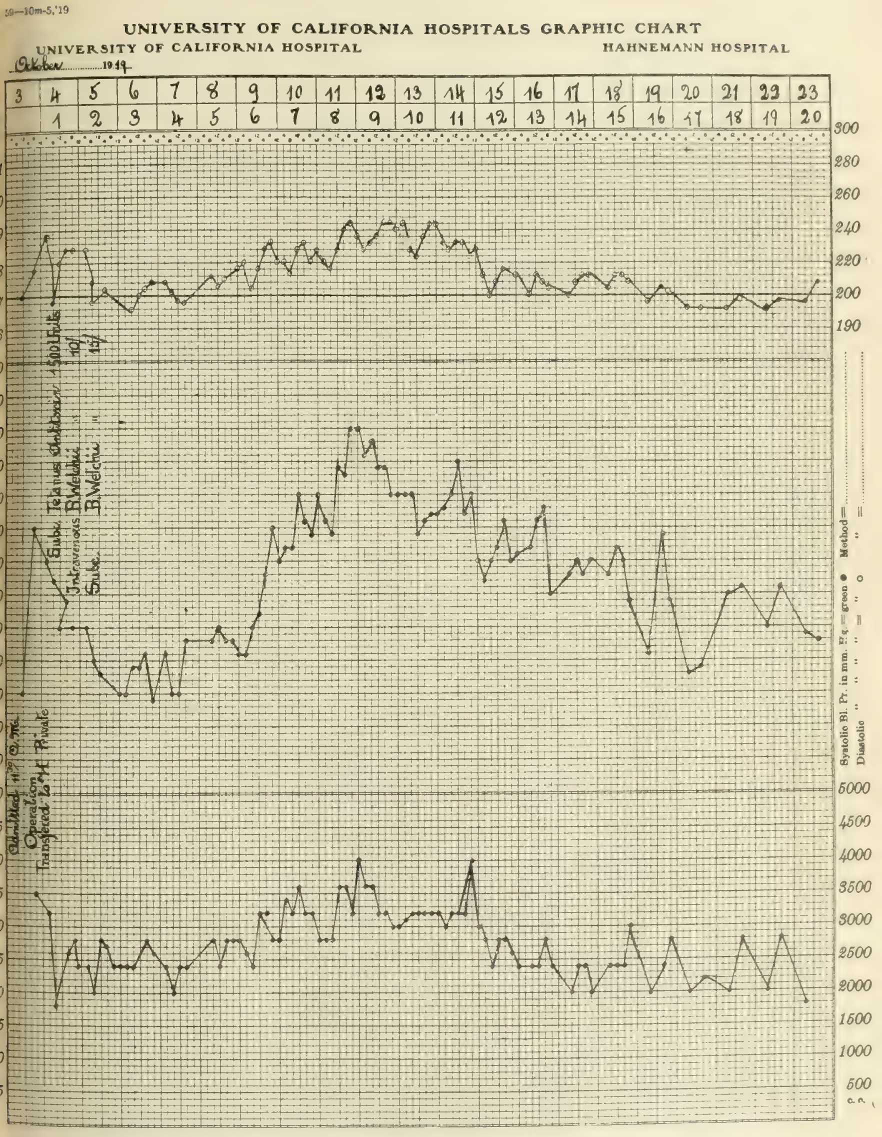

Chart of remarkable tachycardia in a case of gangrene.

containing a clot. The fascia of this area and the muscles below it were blackish red and contained gas. Aside from the gas and color changes, the muscles appeared normal. Nowhere in the forearm was there any pus or exudate. The muscles of the upper arm were but little affected. The fat-containing fascia of this region was, however, markedly gelatinous and contained no gas. The edematous fascia retained its consistency long after section, only a little blood-stained exudate leaking forth.

Twenty-four hours later the arm had lost its rigidity almost entirely, though it had been kept on the ice in the meantime. It is not unlikely that this rapid loss of rigidity was partly due to the escape of the gas and partly to the effect of the bacterial action on the tissues. The muscles were distinctly flabby but not putrid. The connective tissue of the upper arm had lost its gelatinous consistency. A week later, it was found that the area of blackish discoloration was spreading toward the line of amputation, especially near the bone. The arm kept for some time in the refrigerator developed no signs of normal decomposition and the odor remained as before.

MICROSCOPIC AND CULTURAL FINDINGS

At the site of the small perforating wound over the fracture of the ulna were found thick, short, gram-positive, square-ended rods, and in a minority somewhat longer and more slender round-ended rods, not to be easily distinguished from the shorter ones when stained with a thick gentian violet gram stain. A meat medium culture from this region gave an extremely vigorous growth of typical *B. welchii*. In this culture, contaminations were noticeable only after the elimination of *B. welchii* by heat.

A smear from the muscles of the wrist, the region where there was most gas, showed both types of organisms, the heavy rod and the slender rod. A culture gave predominantly *B. welchii*. When this organism was killed, various others were found to be present.

Smears from the palm of the hand showed a clear staining gram-negative rod with rounded ends, and occasional terminal oval spore-bearers. (The thin methyl violet gram stain[3] of the home laboratory was used for this and all the following stains.) A culture on meat medium gave, in twenty hours, gas and a gram-negative rod. This organism produced only a faint pink coloration of the meat particles. The culture seemed to die on exposure to the air, as subcultures showed only a small saprophyte that produced no gas. There was no hint of the presence of *B. welchii*.

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[3] Heller, H. H.: The Etiology of Acute Gangrenous Infections of Animals, J. Infec. Dis. **27**:385 (Nov.) 1920.

Smears from the site of the fracture of the ulna and the muscles above it gave little or no indication of the presence of *B. welchii.* They showed everywhere gram-negative rods, single and in pairs. These were most abundant at the site of the fracture and in the deep red hemorrhagic muscle. The organisms in the muscles in general were vegetative only; those at the site of the fracture were sporulating. The spores were large, oval, usually terminal, with thin walls. They are best described as racket shaped. No bilaterally asymmetrically placed spores were seen. Some bacilli from this region were irregular in their staining, reminding one of the granulose forms of *Vibrion septique.* The edematous fascia of the upper arm contained a very few organisms, some of them sporulating. These were found close to the line of amputation. Cultures taken above the fracture of the ulna gave no Welch bacilli whatever. They contained an organism making a large woolly colony of loose structure with a halo of hairlike radiations. This organism was, so far as it is possible to state, the sporulating one seen on the smears. It proved to be a variety of *Vibrion septique.* A remarkable feature was its greater size in human tissue than in guinea-pig tissue. The eye trained to seeing anaerobes in guinea-pig tissue would find the large racket shaped rods in human tissue more like those of *B. oedematiens* than like *Vibrion septique.* A guinea-pig inoculated in the thigh with 0.5 c.c. of a twenty-four hour culture died in sixteen hours and showed moderate hemorrhagic infiltration of the muscles, little edema, a little gas in the inguinal region, marked injection of the peritoneum, injection of the intestines and of the suprarenals, enlargement of the suprarenals and an invasion of the lungs as shown by congestion and hyperemia. Such lung invasion is rather unusual in the case of *Vibrion septique* strains and is apparently constant in the case of this strain in the guinea-pig body. The lung and liver and peritoneum showed long gram-positive filaments, which are very reluctant to form spores even after forty-eight hours at room temperature; the muscle at the site of inoculation showed forms in all stages of development from purely vegetative, through granulose, swollen forms to sporulating forms. This organism was found also in the wrist and was apparently present in the hand, though cultures failed to show its presence.[4]

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[4] The technic used in the isolation of these strains was simple. Dilution shakes were made in deep peptone liver agar of samples from various portions of the arm. Peptone liver agar is an excellent medium because an exceedingly wide range of anaerobes will grow on it. These shakes were searched carefully and persistently for unusual colony types, and such, when found, were isolated on liver agar and then planted into meat. From the meat, all different types were inoculated into the thigh muscles of guinea-pigs. Details as to the operation of my isolation technic will be found under the title "Principles Concerning the Isolation of Anaerobes," J. Bacteriol. **5**:445 (Sept.) 1921.

There was also, wherever *Vibrion septique* was encountered, an unidentified gram-negative rod that made small dense colonies with short radiations and that belonged to the nonproteolytic group of anaerobic bacteria This organism appeared to be wholly nonpathogenic for the guinea-pig. We shall call it *Bacillus S*.

From the elbow was isolated a third invader that is of interest. It produces lesions much like those caused by certain oedematiens group strains, but culturally it cannot be included in such a group. It possesses distinct though mild proteolytic powers, and in many minor ways differs in its habits from oedematiens group of organisms. This bacillus has marked toxic properties for the guinea-pig and only moderate invasive ones. It usually becomes septicemic in that animal, a fact of interest, because of its invasion in the human case. Considering the number of bacilli present in the animal body at the time of death, this bacillus is far more toxic than the observed strains of other tissue invading groups. Proteolytic action and tissue destruction were not detected in the guinea-pig body probably on account of the sparse proliferation of the bacilli, though a faint proteolytic odor may sometimes be noted. Edema production in connective tissue and muscles is marked; the edema is water-clear. No gas formation has been noted in the animal. Hemolytic action may be entirely absent or may be confined to a spot or two. Injection of the serous surfaces and of the suprarenals is intense. In accordance with a new system of nomenclature,[5] we propose for this bacillus the name *Reglillus progrediens*.

From the region of the wrist, a second new tissue-invading pathogen was isolated. It is a nonproteolytic anaerobe of high saccharolytic powers, and resembles *B. welchii* in its action on tissues. No organism of our acquaintance possesses sufficient characteristics in common with it to warrant its association in the same genus. For it we propose the name *Meyerillus sadowa*. It is an actively invading organism of the muscle-feeding type. It causes considerable muscle and connective tissue destruction in the guinea-pig, and but little gas and edema formations. The intestines are injected and bile-stained, the suprarenals injected and enlarged. The striking lesion is a multitude of hemorrhagic erosions on the stomach lining, resembling leopard spots. The bacilli are densely gram-positive heavy rods with square ends; in the animal body they vary in size and form no chains or spores.

These two species will be described in detail in a future paper.

A strain of sporogenes affinities was isolated from the muscles distal to the fracture; but this type of organism was not present in sufficient numbers to cause any gross evidences of proteolysis.

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5. Heller, H. H.: Suggestions Concerning a Rational Basis for the Classification of the Anaerobic Bacteria, J. Bacteriol. **5**:521 (Nov.) 1921; Certain Genera of the Clostridiaceae, J. Bacteriol. **7**:1 (Jan.) 1922.

No aerobes were found in the material at any point.

On the ninth day after the fracture occurred, exudate from the stump contained leukocytes and was microscopically sterile; but smears from the necrotic areas showed gram-positive sporulating rods resembling *Vibrion septique,* a gram-negative rod, and a few large cocci. Culture showed the presence of *Vibrion septique* and a proteolytic organism.

Much emphasis was laid during the war on the part played by the heavily fertilized soil of France in the causation of gas gangrene. As we here had to do with a much less severe wound than many of those encountered during the war, one containing no earth or other foreign material, and as we found so many species of anaerobes in the wound, an effort was made to investigate the conditions surrounding the accident.

The child had fallen from a high bench, near a street in a suburb of San Francisco, onto dry sandy earth covered with dry grass. Goats were pastured in the neighborhood and had perhaps often crossed the place where the accident occurred; but no manure was demonstrable in the close vicinity. The ground had probably not been cultivated for years. One would not think of it as a particularly dirty spot. Two samples of grass and two of earth were taken to the laboratory and were inoculated into meat medium. All gave a mixture of anaerobes on cultivation. These included heavy gram-positive, short, square-ended rods. All four cultures when inoculated onto milk gave prompt and decided stormy fermentation, which was caused by the short, gram-positive rods, establishing the identity of *B. welchii.* This organism in the meat cultures was killed out by heat, and the resulting meat cultures when twenty hours old were inoculated in 0.5 c.c. doses into the thighs of guinea-pigs. Two of these animals developed a very marked edema, and one of them which had been injected with a culture from grass died in twenty-four hours, and on section, its tissues showed evidence of great proteolytic action. The other was killed after twenty-four hours. The thigh inoculated contained gas and was partially digested. The whole ventral surface to the axillae was covered with a plaque of white serogelatinous edema, a half inch in thickness. This edema was not putrid and contained no gas. These infections were both evidently mixed proteolytic and nonproteolytic ones. *Vibrion septique* may be regarded as having been absent as it almost always causes death in the guinea-pig before the lapse of eighteen hours. Various unidentified anaerobes were cultivated from these guinea-pigs.

It must be remembered that the physician who received the case stated that the arm was wrapped in a towel that was "rather dirty," and that the family of the child lived near a barnyard. Nevertheless, we think that because of the findings in the grass and earth it may be stated that abundant anaerobes may exist on the surface of uncultivated poor sandy soil, and that numbers of these are capable of surviving and multiplying in lacerated muscle, and that some of them may penetrate healthy tissue in proximity to such muscle, even when foreign matter such as soil is not present in the wound.

COMMENT

We have here to do with a mixed anaerobic infection which had as a focus lacerated muscle in the forearm. The organisms found are divisible into groups, as in the accompanying table:

GROUPS OF ORGANISMS

Flora Observed	Proteolytic	Nonproteolytic
Invasive in man in tissue proximal to wound	Reglillus progrediens	Vibrion septique Bacillus S
Invasive in guinea-pig, but not in man		B. welchii Meyerillus sadowa
Not invasive in guinea-pig or properties unknown	Sporogenes type	Small rod producing no gas Gram-negative rod producing little acid Bacillus S

Although the opinion most widely held early in the period of the war was that gas gangrene was a monospecific invasion, caused usually by *B. welchii*, workers who have since made careful bacteriologic studies of their material have emphasized that gangrenous invasions following war wounds are usually polyspecific. The interested reader will find the fullest account of such investigations in Weinberg and Séquin's[6] book and in the report of the Medical Research Committee[7] on anaerobic bacteria and infections. The cases of Weinberg and Séguin are the most carefully worked out and discussed. These authors describe more numerous and more highly polyspecific infections than do other workers This must, in all probability, be attributed to their technic. They use for isolation deep glucose agar shakes, wherein the flora is spread out like a map, and wherein relatively accurate estimates of the number of organisms of different sorts may be made. The British workers have employed surface methods almost entirely. These suffice admirably for *B. welchii* and for *Vibrion septique*, but sometimes fail to show the presence of other types.

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[6] Weinberg, M., and Séquin, P.: La Gangrène gazeuse, Paris, 1918.

[7] Report No. 39 of the Committee on Bacteria and Infections, Medical Research Committee, London, 1919.

The ecology of bacterial organisms entering a sterile territory of rich food supply which has partially but not entirely lost its defensive powers against them is a most interesting study; and it is to be regretted that the exact distribution of these organisms was not more carefully mapped out in this case than it was. For purposes of an ecologic study, the arm investigated may be divided into various zones.

Zone 1. *The Immediate Foyer of the Wound.*—Into this, both aerobes and anaerobes were without doubt introduced. The condition of the tissue was anaerobic; the strict aerobes never developed; the facultatively anaerobic ones were probably all types to which the body had some resistance, and the defensive powers in the damaged tissue were sufficient to destroy them even in the presence of the rapidly developing toxins of the anaerobes. But several types of anaerobic organisms were apparently able to multiply unhindered in this damaged tissue, probably because they were types to which the body was unaccustomed, some of them, types which would probably not have been able to multiply in tissue so little damaged as was the muscle about the fractured bones, if they had not been defended by the toxins of their fellows.

Zone 2. *The Hand.*—The portion distal to the fracture was probably afforded a meager circulation for a considerable period of time after the accident occurred. Invaders capable of traveling along the blood vessels and lymph channels did so and reached the hand. They were the organisms that died after their first cultivation, and the minute rod that produced no gas, perhaps also *Vibrion septique*. These organisms caused the formation of a thick edema in the connective tissue and of a little gas in the region of the small muscles.

Zone 3. *The Wrist.*—As swelling in the region of the fracture increased, the circulation in the distal segment decreased, and some of the active muscle feeders (*B. welchii, Meyerillus sadowa* and the sporogenes-like organism) traveled downward in the muscle, some of which was contused, multiplying extensively and attacking it so severely as to produce gas but no gross tissue destruction, and effectually shutting off the circulation from the entire distal portion of the arm. Organisms found within this region cannot be considered as strongly invasive in their nature, merely because of this invasion of damaged muscle.

Zone 4. *The Section Between the Fracture and the Elbow.*—Here the contest between the tissues and the organisms was acute The blood supply was somewhat subnormal; the tissues were somewhat contused. A reservoir of necrotic, toxin-breeding muscle lay distal to this section. Apparently, only three organisms succeeded in invading this zone, *Vibrion septique,* the mysterious *Bacillus S,* that in the presence of the toxin of *Vibrion septique* was able to avail itself of the same means of distribution as that organism, and a toxic edema-former with slight proteolytic powers (*Reglillus progrediens*). That *B. welchii* was not represented in this zone may surprise some workers. Strains of this type of organism vary in pathogenicity. This was one of moderate pathogenicity for the guinea-pig. The strain *Meyerillus sadowa* was fully as pathogenic for the guinea-pigs as the Welch strain.

Zone 5. *The Upper Arm.*—Here *Vibrion septique* and *Bacillus S* multiplied with a fulminating rapidity and availed themselves of the blood and lymph channels for distribution. At the level of amputation, the blood vessels contained numerous bacilli, and *Vibrion septique,* at least, had invaded the blood stream at the time of the operation. The tissue invasion at this point was slight. After amputation, the bacilli continued to multiply in the tissues of the stump; but the defensive forces were sufficient to prevent extensive necrosis of the tissue and gas formation.

Zone 6. *The Body.*—It is much to be regretted that blood cultures were not made of this case. There must have been a continuous pouring of organisms into the blood stream before the amputation; but it is a question how efficiently the blood defense disposed of them. Klose[8] found that in 60 per cent. of eighty cases of anaerobic wound infection anaerobic organisms were to be cultivated from the blood stream at various stages of the disease, and 51 per cent. of these patients recovered. Therefore, the defense of the body against the persistence of anaerobic bacilli in the blood stream is less than that against anthrax bacilli. On the other hand, the presence of anaerobic bacilli in the blood stream does not indicate so grave a prognosis as does than of anthrax bacilli.

An item of interest lies in the anatomic lesions in the arm. The gelatinous edema of the connective tissue and the black hemorrhages of the muscles were so typical of the lesions produced in the guinea-pig by the oedematiens strain "Joly" that even a very cautious worker would be tempted, especially after finding a large terminal oval spore-bearer and large rods with curved ends, to affirm the presence of that organism. But as far as is ascertainable, a bacillus of the oedematiens group was not present. This type of organism is easily isolated on account of its large colonies. None of the organisms isolated produced in the guinea-pig a black hemorrhagic discoloration like that noted in some of the muscles of the arm. *Vibrion septique* strains produce a less intense coloration in the guinea-pig. The discoloration in the arm muscles must be attributed to the more prolonged action and greater proliferation of *Vibrion septique* organisms in the muscle of a larger and more resistant animal than the guinea-pig. The edema noted in the connective tissue is readily referable to the combined action of the highly toxic edema-producing Reglillus strain and *Vibrion septique.*

The question of the incidence of gas infection in civil life is of interest. Von Hibler[9] lists fifteen strains of *B. welchii* that he isolated from human material. Of these, only two could be called the direct cause of the death of the patient. Both of them were infections after injury. He lists four strains of *Vibrion septique* type isolated by himself; and in three of these cases the organism was demonstrated to be the probable cause of death. He found Novy's bacillus once in a human case, and it was probably the cause of death.

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[8] Klose, F.: Ein Beitrag zur Kenntnis der durch die Gruppe der gas-oedem Bazillen erzeugten anaeroben Wundinfektion, Münch. med. Wchnschr. **64**:295. 1917.

[9] Von Hibler, E.: Untersuchungen über pathogenen Anaeroben, Jena, 1908.

Ghon and Sachs[10] have investigated anaerobic infections occurring in civil life and report various anaerobic invaders.

Cases of anaerobic invasion exclusive of tetanus coming to necropsy in civil life may be classified into two groups. One group is that of patients weakened by sickness or operations who are invaded by their own intestinal bacteria. Such invasions, though they may be, and frequently are, polyspecific, usually have for their chief invader the Welch bacillus. This organism is a common human intestinal saprophyte. Occasionally, Welch bacillus infection follows the injection of drugs into the legs or buttocks. But systemic or local invasions following wound infections, under which head come puerperal infections, especially such invasions as have small foci, are frequently not caused by *B. welchii,* even though the latter organism may be found in the wound. The severity of the tissue destruction and the presence of foreign matter has much to do with the type of invader capable of multiplying in the first hours. The serious wounds caused by high explosives afford far more chance for the growth of *B. welchii* and other muscle feeders than do such cases as the one herewith reported. Welch bacillus infection among animals (except, perhaps, among young sheep) is apparently exceedingly rare, while *Vibrion septique* infection is common. Blackleg infection is found with some frequency among cattle and sheep and oedematiens group infection is noted occasionally among horses. The Medical Research Committee[11] and Sacquépée[12] have miminized the importance of the rôle of the Welch bacillus in war wounds.

The preferred habitat of the anaerobic invaders is of interest. The notable muscle feeders are *B. welchii* and *B. chauvoei.* They may proliferate metastatically, the latter more frequently than the former. *B. welchii* infections may frequently be controlled by excision of infected muscle, while blackleg infections may not. Organisms of the *Vibrion septique* group may feed on muscle, and they frequently do so; but they show a preference for connective tissue and for serous linings, and they also show a remarkable facility for spreading along the lymph and blood channels. Their invasions are, therefore, not to be controlled by excision of individual muscles as are Welch infections. Many of the organisms of the oedematiens group fail to spread rapidly from the site of inoculation and act by means of toxins that advance far before them. Some strains of *B. novyi* may kill guinea-pigs without any invasion at all. Other types, such as the bacillus of Ghon and Mucha and the oedematiens strain "Joly" possess very active invasive powers.

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[10] Ghon, A., and Sachs, M.: Beitrag zur Kenntnis der anaeroben Bakterien des Menchen zur Aetiologie des Gasbrandes. Centralbl. f. Bakteriol. **34**:288, 609; **35**:665; **36**:1,178, 1903.

[11] Medical Research Committee, quoted by Caulfeild, A. H. W.: B. Perfringens: Toxin and Antitoxin Production, J. Inf. Dis. **27**:151 (Aug.) 1920.

[12] Sacquépée, quoted by Caulfeild: Footnote 11, second reference.

Therefore, it is evident that the prognosis of a gangrenous infection in man or animal depends very much on the specific anaerobes that are present in that process.

The value of persistence and careful observation should be emphasized in the investigation of mixed floras. The careful and scientific investigation of a gas gangrene case takes weeks, not hours. The bacteriologist who amiably complies with the clinician's impetuous demand for a diagnosis will usually have to reverse the diagnosis or change it several times. The bacteriologist who contents himself with finding in an invaded tissue Welch bacillus or *Vibrion septique* and who makes no effort to look farther adds nothing to the sum of human knowledge. There are plenty of pathogenic anaerobes still undiscovered. True, it is rare that unusual types invade alone; but unusual pathogens are worth isolating, studying and describing.

For the bacteriologist who is besieged by the clinician, anxious for the life of his patient, the following suggestions may be made. The generous administration to the patient of mixed Welch-vibrion septique-oedematiens serum should be suggested. Smears should be made and cultures taken from affected tissue remote from the wound. Guinea-pigs should be inoculated with fluid drawn aseptically from such tissue. If specific anaerobe antiserums of the three important types are available, three guinea-pigs should be protected, each with two serums in different groupings and all three of them should be inoculated in the thigh with tissue fluid, not with wound exudate, from the patient. In from twelve to eighteen hours, an answer of some value concerning the presence or absence of some one or two of the three important types may be formulated. This procedure is recommended by the British Medical Research Committee.

SUMMARY

The case herewith reported emphasizes the importance of bacteriologic examination early in compound fractures, even in civil life, by one especially skilled in anaerobic work. It shows also that all cases of compound fracture should be under observation in a hospital for at least the first two days after injury.

This case of gas gangrene, which was due to an infected compound fracture of the forearm, was diagnosed before amputation as a Welch bacillus infection.

Four anaerobic bacilli capable of rapid invasion in the guinea-pig body were isolated from the amputated arm. *B. welchii* had not invaded such tissue as was supplied with circulation. The principal invader was *Vibrion septique,* which nearly caused the death of the patient after the amputation. There were two anaerobic bacilli that are guinea-pig invaders which are apparently new. No cocci or aerobic rods were present in the wound or surrounding tissue.

The area about the fracture had not been exposed to very dirty conditions, nor was a visible amount of foreign material introduced into the small wound made by the broken bone. There was, however, comminution of bone which may have had not only a mechanical but a chemical influence on the multiplication of the bacteria that were introduced into the neighborhood of the fracture.

A careful study of the process of invasion by anaerobic organisms in gas gangrene cases, a labor well nigh impossible in most war laboratories, is advisable when time and facilities for the study of anaerobes are available.

Our sincere thanks are due Dr. Karl F. Meyer for his interest in the prosecution and presentation of this work.

Reprinted from the Archives of Surgery
March, 1922, Vol. IV, pp. 470-484

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CHICAGO

STUDIES IN ROOT-CANAL STERILIZATION. III[1]

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(Read before the National Dental Association, Milwaukee, Wisconsin, August 15-19, 1921)

INTRODUCTION

THE study of the problem of sterilization of pulp canals falls into three main divisions. The first is concerned with the gross and microscopic anatomy of the dentin and cementum; the second, with methods of treatment, and the third, with an analysis of the results from a clinical and bacteriological standpoint. A contribution to the microscopic anatomy was offered in a report upon the organic matrices of dentin and cementum and in it a method was described by which it is possible to separate and differentiate the organic matrices of the structures concerned (1). This separation between the two structures, as has been previously observed, indicates that dentin matrix and cementum matrix are without demonstrable connection. Since it has been proved by others that the ground section of a calcified specimen presents no conclusive evidence of direct communication it follows that with the exception of accessory foramina, there have been no experimental data submitted which would suggest the existence of this anatomical characteristic. These researches point, on the other hand, to the fact that the structures do not possess interlocking fibers in the organic matrices, or communicating channels in the calcified specimen. It follows, then, that antiseptics or fluids which are placed in the pulp canal for the purpose of sterilization are probably not directly subject to dilution and removal by the collateral circulation of the peridental membrane. Osmosis may tend toward such a dilution and the possibility of this action has been discussed in a previous article.

The extent of penetration of fluids in general and of certain antiseptic solutions in particular was demonstrated in a second article (2). Included in this discussion was the description of a new application of a solution of crystal violet and brilliant green. The advantages and disadvantages which this solution possesses were compared with other antiseptics and in particular with the silver treatment as advocated by Dr. Howe.

The next part of the problem deals with the relative efficiency of the antiseptics employed and may be summarized by the question, What success may clinicians expect from the treatment of infected teeth and how may that success best be measured?

This paper reports the results obtained from bacteriological tests following the treatment of infected pulp canals. It constitutes, in fact, a preliminary survey of two problems. First, a consideration of different sterilization methods adopted in this class of dental operation, and second, a comparison of clinical and laboratory results obtained with a few selected antiseptics. The work reported is based on clinical and laboratory records of 127 cases presented at the dental clinic of the College of Dentistry, University of California.

At the beginning of the experiment it was sought to determine whether it

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[1] From the Research Laboratories of the George Williams Hooper Foundation for Medical Research and of the College of Dentistry, University of California. Aided by grant from the National Dental Association

Reprinted from The Journal of the National Dental Association, March, 1922.

was possible to treat a pulp canal in the clinic in such a manner as to render it sterile. Altho our present technic has been improved by the correlation and interpretation of laboratory data, the clinical findings have not been sufficiently tabulated and analyzed. It is this tabulation and analysis which is presented for your consideration. The research of Cevey reported from Zurich is confirmed, but the list of antiseptics employed by him has been considerably extended.

In seeking for a standard clinical method of procedure, it is at once apparent that the factors in the personal equation, or the habits and technic of the operator, are variable and fluctuating. Clinical procedure is never absolute. It is relative, not only from the standpoint of the operator, but also when compared with the work of others. It is not claimed that the operative details which were assigned have any particular virtue over others. They were adopted as a standard because it was necessary, when beginning experimental work, to formulate uniform procedures which will render reliable results, and which admit of clinical adoption.

After the assignment of the patient to the student a differential diagnosis of the pathological condition was made by the operator and then corrected or confirmed by the instructor. Following this the designated treatment for the case was instituted and continued under careful supervision. At the conclusion of the treatment the pulp canal was tested for sterility, as described later. The sterilized rubber dam was adjusted and the exposed area, both dam and teeth, washed several times with 70 per cent alcohol. The tooth under observation was then carefully opened, the dressing removed and the canals dried with sterilized cotton points.

It has been reported by Pond (3) and others that the cotton points supplied to the profession in "aseptic" (?) sealed cartons are usually contaminated. Altho the contamination may be from an organism of a non-pathogenic type, it is nevertheless incorrect to assume that such a dressing is aseptic. All dressings used were sterilized in test-tubes placed in the Rochester type of steam sterilizer.

In the treatment of an individual case, a score or more of these points were shaken from a test-tube into a sterile petri dish, a procedure which obviated the necessity of inserting an instrument into the tube. For if an instrument is used to carry the points from a test-tube to the canal directly, there is always the probability of contamination of the entire stock. By placing sufficient points for the case at hand in the petri dish, this risk is reduced to a minimum. The dressing is carried from this container to the tooth with treatment pliers. These are first dipped in alcohol and then flamed before picking up the cotton point. The cover of the petri dish should not be removed entirely, but should be raised just enough to permit access to the sterilized canal dressing in the dish. In preparing the pulp canal for the bacteriological test, it was first carefully dried, a fresh *dry* sterile point inserted in the cavity, and the tooth sealed in the following manner.

A small pellet of sterilized cotton was forced with pressure into the opening of the canal. Following this the apical portion of the pulp chamber was sealed with baseplate gutta-percha. The cavity was moistened with a little chloropercha, and warm baseplate gutta-percha sealed into the apical half of the pulp chamber. The remainder of the cavity is filled with Ames' crown and bridge cement. It has been found that by following this procedure more uniform results are obtained, for even the lower central incisors, teeth with small pulp chambers, may be handled in this manner. The following day the tooth was again opened under the sterile conditions described above, and the cotton removed from the canal. A series of culture tubes, containing glucose blood broth were placed at the operator's left and the cotton point carried immediately from the pulp canal to the tube. The canal was again inspected for moisture and the second point inserted for a few moments. Usually two tests of each canal were conducted but in some cases this was not feasible.

The tube containing the point was incubated and the specimen examined microscopically, in accordance with the usual bacteriological technic. In cases where organisms were found, the material was transplanted to blood agar plates and the cultural characteristics determined.

The tables indicate the results of this experiment. They summarize the pathological condition, and the method of treating the individual cases. Frequently it was necessary to repeat the treatment and this repetition has been indicated by a numerical reference. In some instances it will be noted that the infected canal, even tho the treatment was repeated, could not be rendered sterile. The canal, however, was perfectly dry and showed no clinical sign of infection. These canals were filled in spite of the fact that it was impossible with our technic, at least, to secure a sterile test. On two occasions this practice was followed by the development of an acute apical abscess.

The administration of a large clinic is different from that of a private practice, for the reason that the patients cannot obtain the same professional attention accorded under less public conditions. Appointments are frequently cancelled and valuable time lost. It has been necessary, therefore, on some occasions to use more than one antiseptic in a canal. For example, in Case 22, Table I, a putrescent pulp was treated successively with formo-cresol, dichloramine-T, and crystal violet and brilliant green. The reasons necessitating this procedure were those over which the

TABLE I

Pulp Canals Sterile After First Series of Treatment

No.	*Clinical Record*	*Therapy*	*Result*
93	Putrescent pulp	Beechwood creosote	Canals fiilled
94	Pulp exposed and extirpated by pressure anesthesia	Phenol	" "
96	Dry gangrene	Dichloramine-T	" "
105	Pulp exposure	"	" "
30	" "	"	" "
83	Putrescent pulp	Formo-cresol	" "
88	Partly putrescent pulp	Dichloramine-T	Extracted
89	" " "	"	"
90	" " "	"	"
95	Pulp exposed	Beechwood creosote	Canals filled
28	Putrescent pulp	Dichloramine-T	" "
47	Acute apical abscess	"	" "
11	No record	"	" "
12	" "	"	" "
15	Acute apical abscess	"	" "
18	Chronic apical abscess	"	" "
19	" " "	"	" "
20	" " "	"	" "
67	" " "	"	" "
68	" " "	"	" "
45	" " "	"	" "
56	" " "	"	" "
57	" " "	"	" "
58	" " "	"	" "
82	Acute apical abscess	Dichloramine-T	Canals filled
		"	
84	Chronic apical abscess	Beechwood creosote	" "

No.	*Clinical Record*	*Therapy*	*Result*
85	Chronic apical Abscess	Beechwood creosote Dichloramine-T	Canals filled “ “
101	“ “ “	Beechwood creosote Dichloramine-T Formo-creosol	Formo-cresol dressing in canal. No filling
102	“ “ “	Beechwood creosote Dichloramine-T Formo-creosol	Canal filled
106	“ “ “	Crystal violet and brilliant green	“ “
107	“ “ “	Crystal violet and brilliant green	“ “
108	“ “ “	Crystal violet and brilliant green	“ “
120	“ “ “	Beechwood creosote	“ “
125	Unfilled canal Mumifying paste	Dichloramine-T	“ “
126	Unfilled canal	“	“ “
121	Putrescent pulp	“	“ “
123	“ “	Beechwood creosote Dichloramine-T	“ “
124	Pulpitis	Dichloramine-T	“ “
128	Exposed pulp	“	“ “
129	Putrescent pulp	“	“ “
64	“ “	Beechwood creosote	“ “
62	Extirpated pulp	“ “ Dichloramine-T	“ “
46	Devitalized pulp As_2O_3 fiber	Beechwood creosote Dichloramine-T	“ “
41	Extirpated pulp	Beechwood creosote	“ “
137	Pulp exposure	Eugenol Dichloramine-T	“ “
42	Putrescent pulp	Beechwood creosote	“ “
138	Chronic apical abscess	Dichloramine-T	“ “
139	Dry gangrene of pulp	Beechwood creosote Dichloramine-T	Canals filled
55	Exposed pulp devitalized by As_2O_3 fiber	“	“ “
32	Putrescent pulp	“	“ “

college had no control. In studying these tables it must be borne in mind that the efficiency of dichloramine-T continues only while chlorine is being liberated from the compound. This antiseptic was applied four separate times twenty-four hours apart (4). If the dichloramine-T remained longer than twenty-four hours, the procedure was repeated. There were other instances in which the treatment was recommenced on account of faulty technic of the student. His operative skill is never as highly developed as that of an experienced operator; his method of handling instruments and of handling clinic materials is sometimes clumsy. But on the other hand, it is probable that his training in bacteriological technic is better than that of the average dentist. It is apparent, therefore, that there was a greater necessity for the re-treatment of canals than would be necessary in private practice with an experienced operator.

In Table I are grouped those cases which after the first series of treatments were found sterile by laboratory test. The clinical record includes acute and chronic apical abcess as well as putrescent and gangerenous pulp conditions.

Experiments 88, 89, and 90 deserve comment. In this particular case, a partly putrescent pulp was causing severe pain and a mandibular injection for removal of the residual hypersensitive pulp filaments was unsuccessful. After a series of treatments with dichloramine-

T followed by eugenol, the inflammation subsided to such an extent that devitalizing fiber was applied. Subsequently the remaining pulp stumps were removed and the canals prepared for filling. A radiograph taken at this time showed that one of the canals had not been opened entirely to the apex. Further attempts at reaming the canal were unsuccessful. A second radiograph taken with the diagnostic wires in place was misinterpreted and a diagnosis of a perforated root was made. On extraction of the tooth the roots were carefully examined but no perforation could be found.

Table II summarizes the pathology, treatment, and the result of cases similar to those in Table I.

Since none of these proved sterile after the first test, the therapy was repeated. A numerical reference in the third column has been given which furnishes an index to the subsequent data found in Tables II and IV.

Altho the majority of cases cleared up satisfactorily after this renewed attempt at sterilization, there were some of these which apparently remained infected. These are summarized in Table IV.

With the permission of the patient the canals were filled and the patient in-

TABLE II

Pulp Canals Infected After First Series of Treatments

No	*Clinical Record*	*Therapy*	*Result*
70	Acute apical abscess	Dichloramine-T	Treatment repeated 98
71	" " "	"	" " 99
72	" " "	"	" " 100
34	Dry gangrene	Beechwood creosote	" " 34a
73	Chronic apical abscess	Crystal violet and brilliant green	" " 73a
74	Putrescent pulp	Crystal violet and brilliant green	" " 74a
40	Infected pulp	Eugenol	" " 40a
54	" "	Beechwood creosote Crystal violet and brilliant green	" " 54a
43	" "	Dichloramine-T	" " 44
22	Putrescent pulp	Formo-creosol Dichloramine-T Crystal violet and brilliant green	" " 29 & 29x
21	Chronic apical abscess	Dichloramine-T Beechwood creosote	" " 23
13	Putrescent pulp acute pericementitis	Dichloramine-T Beechwood creosote 11/17/20.-1/20/21 Dichloramine-T 1/20/21 - 1/24/21	" " 13a
14	Putrescent pulp acute pericementitis	Dichloramine-T Beechwood creosote Dichloramine-T	" " 14a
80	Putrescent pulp	"	" " 80a
97	Pulp exposure	"	" " 97a
86	Putrescent pulp	" Beechwood creosote Formo-cresol	" " 86a
103	Pulp exposed	Dichloramine-T	" " 103a
104	" "	"	" " 104a
38	Acute apical abscess	Dichloramine-T	Patient did not return for further treatment

No.	*Clinical Record*	***Therapy***	*Result*
127	Unfilled canal	Dichloramine-T	Treatment repeated 127a
1	No record	Beechwood creosote	" " 1a
2	" "	" "	" " 2a
3	" "	" "	" " 3a
17	" "	Dichloramine-T	" " 17a
66	" "	"	" " 69
49	" "	Beechwood creosote	" " 65
65	" "	" "	" " 81
76	" "	" "	" " 87
75	" "	Dichloramine-T	" " 91
111	" "	"	" "
112	" "	"	" "
113	" "	"	" "
114	" "	Beechwood creosote	" "
115	" "	" "	" "

TABLE III

Pulp Canals Sterile After Second Series of Treatments

No.	*Clinical Record*	***Therapy***	***Result***
80a	Repetition of 80	Crystal violet and brilliant green	Canals filled
97a	" " 97	Dichloramine-T Crystal violet and brilliant green	" "
86a	" " 86	Dichloramine-T	" "
103a	" " 103	" Crystal violet and brilliant green	" "
104a	" " 104	Dichloramine-T Crystal violet and brilliant green	" "
127a	" " 127	Dichloramine-T	" "
98	" " 70	"	" "
99	" " 71	"	" "
100	" " 72	"	" "
34a	" " 34	"	" "
73a	" " 73	Crystal violet and brilliant green	" "
74a	" " 74	Crystal violet and brilliant green	" "
40a	" " 40	Dichloramine-T	" "
44	" " 43	"	" "
29	" " 22	"	" "
29x	Check test of 29	"	" "
23	Repetition of 21	" Crystal violet and brilliant green	" "
13a	" " 13	Dichloramine-T	" "
14a	" " 14	"	" "
17a	" " 17	"	" "
69	" " 66	"	" "
81	Repetition of 49	Dichloramine-T	Canals filled
81a	" " 65	"	" "
87	" " 76	Beechwood creosote	" "
91	" " 75	Dichloramine-T	" "
114a	" " 114	"	" "
115a	" " 115	"	" "
1a	" " 1	"	" "
2a	" " 2	"	" "
3a	" " 3	"	" "

TABLE IV

Pulp Canals From Which No Sterile Test Could Be Obtained

No.	*Clinical Record*	*Therapy*	*Result*
51	Imperfect canal filling	Dichloramine-T	Canals filled
52	" " "	"	" "
53	" " "	"	" "
59	" " "	"	" "
48	Putrescent pulp	"	" "
54a	Repetition of 54	Beechwood creosote Crystal violet and brilliant green	Treatment repeated 61
61	" " 54a	Dichloramine-T	Canals filled Acute apical abcess developed
33	Putrescent pulp	" Beechwood creosote	Canals filled
35	Chronic apical abscess	Dichloramine-T	Tooth extracted
36	" " "	"	" "
37	" " "	Formo-cresol	" "

TABLE V

Infected Pulp Canals

Incomplete Clinical and Laboratory Records

No.	*Clinical Record*	*Therapy*	*Result*
38	Acute apical abscess	Dichloramine-T	Patient did not return for root-canal filling
39	Canals previously filled with iodoform paste No clinical symptoms	"	" "

structed to return for observation and for further radiographic diagnosis. In those instances where it was found inadvisable to fill the canal, the tooth was extracted.

It is realized, of course, that this is only a step taken toward the study of root-canal sterilization. One of the points yet to be considered is this. After a sterilized root canal has been filled properly what condition may we expect to find at the end of six months or a year when we remove the root-canal filling? Will the area remain sterile during this interval of time or will organisms from other localities have found favorable opportunity in this region for growth? Is tissue resistance lowered in the periapical region and if so, what is the effect of this lack of tone? Theoretically the canals should be sterile and the experimental work was designated to prove or disprove this point. Clinically, however, we are still in the dark. We do not know the final result. Our lack of authentic data on this phase of the problem emphasizes the necessity of maintaining case records which show both treatments and clinical conditions at regular intervals. Whenever it becomes necessary to institute therapeutic measures, appropriate notations should be carefully recorded and checked from time to time.

In all there have been 127 cases reported. Of this number 10 per cent (Tables IV and V) remained infected after repeated treatments; 39.4 per cent were sterilized successfully the first time; 26.8 per cent were infected after the first series of treatments, and 23.6 per cent were found sterile after the repetition of the clinical procedure.

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Reprint from the California State Journal of Medicine.
January, 1922

A LABORATORY AND CLINICAL STUDY OF THE BACTERICIDAL ACTION OF SOLUTIONS OF RADIUM EMANATION

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From the Radium Laboratories of the University of California Hospital; the Research Laboratories of the George Williams Hooper Foundation for Medical Research and of the College of Dentistry, University of California, San Francisco.

Recent observations upon the use of radium emanation indicate that there is a possibility of employing this substance in the treatment of chronic dental abscesses. Until further laboratory data can be obtained, it appears advisable to mention briefly the results thus far recorded, with the hope that other workers in this field may be sufficiently interested to verify some of the conclusions.

The bactericidal action of radium has been studied particularly by Lequeux and Chrome,[1] and Iridell and Minett.[2] In their experiments they exposed different organisms to radium emanation for varying periods of time with a noticeable inhibitory effect upon the growth of cultures of streptococcus. Although the author has not repeated the experiments referred to above, his clinical findings are in apparent accord with the deductions offered. Bacteriological examinations of the treated canals indicated sterility in 85 per cent of the cases. In fact, the summary presented points to the possibility of a new application of radium in the treatment of certain diseased conditions.

The apparent success which has attended the radium therapy of skin lesions suggested the advisability of developing a modification of the present method of application which would make possible the study of the effects produced by this agent on chronic dental apical abscesses. A summary of nearly a year's observation of clinical cases shows that lesions of this type yield readily to the new therapy, and that up to the present time no untoward effects of the radium treatment have been observed.

Through the courtesy of Dr. Howard Morrow, Dr. Laurence Taussig, and Mr. L. B. Clark, it has been possible for the author to secure capillary tubes containing radium emanation. These tubes had been used in the skin clinic and eye clinic for various purposes until the "de-emanation" had proceeded to such an extent that further application for this work was purposeless. Although the degree of radiation is recorded in the tables in terms of millicuries, the measurement of the Beta radiation from radium *D* and *E* has not been made.

Radium emanation or Niton is a gas, the disintegration products of which are solids. It is a solution of the solids which is being employed. That this solution contains alpha, beta and gamma rays is known, but the effect upon diseased tissue is perhaps due to the predominance of the beta radiation coming from radium *D* and *E*. It may be recalled that Tarnowsky[3] quotes Hektoen to the effect that radium "emanation" inhibits phagocytosis and, hence, lowers the natural resistance of the affected part; but in these experiments I have employed, as already stated, a preponderance of the soft beta rays.

METHOD OF PROCEDURE

A small amount of Ringer's solution may be rendered radio-active by crushing in it a capillary tube containing a known quantity of radium emanation. This operation is conveniently accomplished by placing the tubes in a sterilized mortar of about 20 cc capacity, covering the tubes with Ringer's solution and then crushing them. The preparation is transferred to a suitable container and carried to the operating-room. On account of the rapidity with which the radio-activity of this solution diminishes, it was found advisable in this research to crush the tubes at the exact time at which the experiment was performed, but no more than an hour was permitted to elapse between the preparation of the solution and its application in the clinic or laboratory.

The radio-active liquid was carried to the root canals of the tooth by means of J. & J. sterilized cotton points. These were sealed in the canals for a period of from one to four days. Subsequently the treatment was removed, the pulp canals tested for sterility, and finally filled. With one possible exception, there has been no evidence of succeeding soreness or pain. The exception referred to is one in which the pericementitis resulted more probably from mechanical trauma incident to the previous curetting of the canal than to the action of the radium itself. The amount of solution carried to the tooth averaged 0.05 cc.; in this there was approximately 0.0630 millicuries of radium emanation. The variation in the dosage is noted in the appended table.

Table I

Millicuries of tubes *	Millicuries per cc. of solution	Amount placed in tooth
6.0	1.20 per cc.	.060 millicuries
7.9	1.58 " "	.079 "
5.5	1.10 " "	.055 "
4.6	0.92 " "	.046 "
9.1	1.82 " "	.091 "
7.5	1.50 " "	.075 "
6.3	1.26 " "	.063 "
5.3	1.06 " "	.053 "
13.8	2.76 " "	.138 "

Table II

Summary of Twenty-seven Cases

Clinical condition	Treatment	Results
Chronic apical abscess.	Radium applied four days.	Canals filled. No unfavorable symptoms after ten months.
Infected pulp with acute pericementitis.	Radium applied seven days.	Pericementitis very severe. Tooth extracted.
Chronic apical abscess of long standing.	Radium applied seven days, removed. Second treatment given.	Canals filled and no unfavorable symptoms after ten months.
Acute abscess.	Two treatments of radium of three days each.	Canals filled. No unfavorable symptoms after six months.
Chronic apical abscess.	Two treatments of radium of three days each.	Canals filled. No unfavorable symptoms after six months.

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* Not freshly prepared. These values were computed from tubes which originally contained a radio-activity varying from 50 to 100 millicuries.

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RADIUM THERAPY IN PERIAPICAL INFECTIONS[1]

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(Read before the American Dental Association, Los Angeles, California, July 17-21, 1922)

THIS paper supplements a preliminary communication published earlier in the year (1). The original conclusions made therein have been confirmed, namely, that solutions of the products of radium emanation possess distinct bactericidal properties and are useful in the treatment of periapical infections. Additional data are presented but the analysis of them clearly indicates that with the present day knowledge it is impossible to state definitely and conclusively why the emanations produce such characteristic effects. This report should therefore be considered merely as a statement of progress. However, it may be advisable in this connection to summarize briefly the theoretical considerations which justify the application of radium in the treatment of dental infections.

A review of the literature dealing with radium shows that its bactericidal action has been described by a number of workers, although nothing definite is published with regard to the clinical application of this property. As early as 1909, Iridell and Minett (2) observed that radium exerts an inhibitory influence on pathogenic and non-pathogenic organisms. Ten years later Lequeux and Chrome (3) confirmed this in their report upon the action of radium on bacteria. In their experiments they exposed different organisms to radium emanation for varying periods of time. The emanation exerted a noticeable inhibitory effect upon the growth of cultures of streptococcus.

In 1919 the author received a supply of ampules containing radium chloride and sodium hexavanadate. He sought to determine the efficacy of this solution in the treatment of gingival and periapical lesions. The reagent was packed in sterile glass ampules containing "2 micrograms of radium element as chloride and 50 milligrams of V205 in the form of sodium hexavanadate in 2 ccs. of sterile normal physiological salt solution." Although the results of a preliminary series of tests conducted in the dental clinic of the University of California were disappointing, they suggested the possibility of using other radium preparations in the treatment of periapical infections.

The evident success which has attended the radium therapy of skin lesions induced the writer to devise a modification of the present method of application which would make possible the study of the action produced by this agent on acute and chronic dental apical abscesses. Observation of clinical cases, extending over two years, has shown that lesions of this type readily yield to the new method of treatment, and up to the present time no untoward effects following the use of radium have been observed.

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[1] From the Radium Laboratories of the University of California Hospital; The Laboratories of the George Williams Hooper Foundation for Medical Research, and the College of Dentistry, University of California. Aided by grants from the American Dental Association, and from the Board of Research, University of California.

Reprinted from The Journal of the American Dental Association, December, 1922.

Through the courtesy of Dr. Howard Morrow, Dr. Laurence Taussig, and Mr. L. B. Clark, it has been possible for the author to secure capillary tubes containing radium emanation. These tubes had been used in the skin clinic and eye clinic for various purposes until the "de-emanation" had proceeded to such an extent that further application for this work was purposeless. Although the degree of radiation is recorded in the tables in terms of millicuries, the measurement of the beta radiation from radium D and E has not been made.[2]

Radium emanation or niton is a *gas*, the disintegration products of which are *solids*. It is a solution of these solids which is being employed. Tubes containing the emanation are customarily measured in terms of gamma radiation. As each preparation grows older it suffers a slow decomposition so that the hard gamma rays grow less each day, until they finally become an immeasurable quantity. At this time the conversion of the radio active substance to its next lower disintegration product has been partly completed.[2] These newer products, principally radium D and E, give off what is termed the beta rays. Since the gamma radiation has become practically nil, the effects produced are in all probability due to the influence of the beta ray. It may be recalled that Tarnowsky (4) quotes Hektoen to the effect that radium "emanation" in hibits phagocytosis and hence lowers the natural resistance of the affected part; but in these experiments there has been employed, as already stated, a preponderance of the beta rays.

METHOD OF APPLYING THE PRODUCTS OF RADIUM EMANATION TO THE DISEASED TOOTH

It has been found practical to apply the radium in the form of a solution The latter is prepared as follows: A radium tube is placed in a sterilized mortar of about 20 cc. capacity, covered with Ringer's solution, and then crushed with a pestle. On account of the rapidity with which the radio-activity of this solution diminishes, it is advisable to crush the tubes immediately before use.

The radio-active liquid is carried to the pulp canals of the tooth by means of sterilized cotton points. These points are sealed in the canals for a period of from one to four days. Subsequently the dressing is removed, the pulp canals tested for sterility, and finally filled.

The sterility tests of the root-canals are conducted in the following manner The tooth is opened under the sterilized rubber-dam and the crown and adjacen areas flooded with alcohol, thoroughly painted with a one per cent solution o crystal violet—brilliant green, and dried with hot air. The cavity is exposed and the cotton points which originally carried the radium solution are discarded No attempt is made at this time to desiccate the canal, but the excess moistur is removed by repeated applications o fresh sterile cotton points. Following this another point is placed in the canal and sealed under both base plate gutta-percha and crown and bridge cement. The gutta-percha is used in the pulp chamber for the reason that it may be sterilized by dry heat immediately before it is placed in the cavity. This filling of itself is not sufficient, for it may be recalled that under the stress of mastication, the gutta-percha tends to pull away from the walls of the cavity and thus permit reinfection from the flora of the buccal cavity. This discrepancy is overcome however by supplementing the partial filling by one of crown and bridge cement. The cotton point is allowed to remain twenty-four hours and then removed in the same aseptic manner in which it was placed. It is dropped immediately in a tube of culture media, consisting of glucose veal infusion sheep's blood broth. This has been found to be particularly favorable for the germination of the organisms usually identified with these lesions. The property of delayed growth of the streptococcus group is of course well known, but in these clinical observations, it has been found practical to incubate from 24 to 96 hours.

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[2]The term millicurie may need definition. Radium, like other radio active elements, is constantly undergoing disintegration, by breaking up continuously into elements of a lower atomic weight. The *first* disintegration product of radium is radium emanation or niton. The unit which expresses the quantity of niton is called a curie, and this unit represents the amount of niton in equilibrium with one gram of radium. The working unit, however, is one-thousandth of this amount, or a millicurie. The second disintegration derivative of radium is radium A. The third, radium, B, and so on until polonium, and finally presumably lead is obtained. The life of these many decomposition products covers a wide range, and varies in fact from a few seconds to a number of years. The small capillary tubes which are being used contain radium emanation, together with the elements of lower atomic weight, namely, radium A, B, C and D. These are the substances used in this research. All of the derivatives of radium emanation are in a state of transitory equilibrium with one another. That is, they are continually decomposing into their next lower disintegration products. Since radium D is derived from previous short time substances, and since its period of disintegration is longer, it is evident that it exists in preponderance in the tube. In addition to radium D, there are presumably minute quantities of radium E and F (or polonium).

With two possible exceptions, there has been no evidence of succeeding soreness or pain. One of the exceptions referred to was one in which the pericementitis resulted more probably from mechanical trauma incident to the previous curetting of the canal than to the action of the radium itself. The amount of solution carried to the tooth averaged 0.05 cc; in which there were approximately 0.0630 millicuries of products of radium emanation.

EXPERIMENTAL DATA

For the sake of brevity the correlated data have been presented in tabular form.

Taken in the order in which this information was compiled the primary point of interest is that of dosage. As has already been stated the strength of the various solutions has been measured in millicuries of gamma radiation, and although this serves as a basis of comparison, it does not give an exact indication of the amount of beta ray contained in the different solutions.

A brief synopsis of several case records serves to indicate the variations in the pathology, age of patient, etiology, treatment and clinical result. In this connection, it may be stated that some of the cases are students at this institution and radiographic evidence covering at least four years will be presented in due time.

The variation in dosage is noted in the appended table:

Millicuries of tubes	Millicuries per cc. of solution	Amount placed in tooth
6.0	1.20 per cc.	.060 millicuries
7.9	1.58 per cc.	.079 millicuries
5.5	1.10 per cc.	.055 millicuries
4.6	0.92 per cc.	.046 millicuries
9.1	1.82 per cc.	.091 millicuries
7.5	1.50 per cc.	.075 millicuries
6.3	1.26 per cc.	.063 millicuries
5.3	1.06 per cc.	.053 millicuries
13.8	2.76 per cc.	.138 millicuries

The following synopsis of selected case records will help to describe not only the varied pathological conditions studied, but also the extent of treatment which was occasionally found necessary.

No.	Clinical Condition	Etiology	Treatment	Result
486	**Dry gangrene of pulp. Upper left cuspid. Patient aged 20.**	Mechanical trauma from fall some years previous.	Radium treatment 2-10-22. Sterile test after 48 hours incubation 2-14.	**Canals filled 2-22 Check up O. K. 6-29.**
4344	**Chronic apical abscess. Upper left central incisor. Age of patient between 40 and 45.**	Caries. Previous nine weeks treatment outside of college unsuccessful. Root-canal reamed too large at apex for ideal filling.	Radium treatment 3-2. Sterile test after 24 hours incubation 3-7.	**Canal filled 3-11-22. Radiograph showed over filling. Alveolus opened at apex and excess gutta-percha removed. Radiograph shows apparently normal tissue 7-3-22.**

No.	Clinical Condition	Etiology	Treatment	Result
5178	Chronic apical abscess. Upper left second bicuspid. Age of patient between 50 and 55.	Caries.	Imperfect root-canal filling removed. Radium 4-13-22. Sterile test after 72 hours incubation 4-15.	Canal filled 4-17-22. Tooth normal 6-30.
953	Chronic abscess with fistula at bifurcation of roots. Lower right first molar. Patient aged 20.	Caries.	Fistula lanced 4-11 Dichloramin T 4-12 Radium 4-13 Radium 4-14 Creosote and viogen in fistula 4-15 Radium 4-20 Sterile test after 48 hours incubation.	Canal filled 4-23.
3963	Chronic apical abscess, drainage thru canal. Upper left central incisor. Patient aged 12.	Trauma in which portion of crown was broken off.	Dichloramin T creosote from 3-28 to 4-20 Radium 4-20 Sterile test after 24 hours incubation 5-6	Canal filled 5-8
RESUME				
	Age of patient varies from 12 to 55. Conditions range from acute abscesses to chronic abscesses with and without fistulae.	Infective.	Some cases treated without any other drug having been used. Others have had many previous treatments without success. Canals found to be sterile in 85% of cases.	Canals filled. No teeth have shown adverse clinical symptoms to date.

Discussion

There are certain outstanding features of the treatment which merit additional discussion.

In many instances tubes have been used in which de-emanation had proceeded to such a degree that it was not possible to obtain a reading by the electroscope. These have given similar results in so far as bactericidal action is concerned, but they have not caused the soreness which sometimes accompanies the use of solutions containing a recorded amount of gamma rays.

In general the cases which have been selected have been those in which previous treatments have been without satisfactory result. Dichloramin T, formocresol, beechwood creosote, phenol, and other antiseptics of a similar nature had been used without producing a noticeable change in condition. In fact, the greater majority of the cases carried a history of unsuccessful treatment. Most of these yielded to the action of the radio active solution. It might be argued that the results which were obtained by the radium therapy were not due to this agent, but were due to the previous treatments of other drugs. Such a view hardly coincides with the facts of the case, however, for when the patients were referred to the clinician for further work, they had been classed as hopeless in that other therapeutic agents had failed to produce any observable change in the condition.

Sterile pulp canals have not been obtained in every case, neither have perfect root-canal fillings been placed in each instance, but in spite of this fact, no teeth have been lost as a result of radium therapy. Failures in obtaining sterility of the pulp canal are difficult to trace and almost any number of reasons may be advanced to account for this lack of one hundred per cent efficiency. Other methods of therapy have likewise indicated that a successful operation may not always be expected. But the fact remains that sterile tests have been obtained more often with *this* treatment than in similar experiments with other antiseptic agents.

During the last two years there has accumulated radiographic, as well as clinical evidence to show that healing in the apical area is progressing to a satisfactory degree. Clinical examination shows a tooth performing its function properly and without discomfort or distress to the patient.

The precautionary measures which are to be observed are naturally those referring to dosage and technic of application. Severe radium burns resulted in monkeys when emanation tubes containing eighteen millicuries were sealed into pulp canals of central incisors. These burns were so extreme that the hard palate and orbit were involved. It is believed to be impracticable to use the radium except as a solution of the solids. The tubes should be thoroughly crushed under Ringer's solution exactly at the moment of use, and it has been customary to have three or four patients prepared and ready at one time in order to secure the greatest economy of material. The limited availability of de-emanated tubes except at emanation laboratories is a serious drawback to an extensive application of this therapy. However it is planned, if funds become available, to further extend these researches to include not only additional data upon dosage, but also to report upon the application of other radio-active preparations which may be found to be more plentfully distributed.

Conclusions

1. A method of preparing a radio-active solution is described.

2. This preparation is useful in the treatment of chronic dental apical abscesses.

3. Sterility of the pulp canal by means of this therapy is obtained in eighty-five per cent of the cases.

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METHOD FOR PREPARING BLUE CELLULOID INJECTION MATERIAL*

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This note refers to those anilin dyes which may be used for coloring specimens prepared by the celluloid injection method.

In a study of the anatomic relations of the salivary glands, it became of interest to inject simultaneously the gland substance through the excretory duct, and the arterial blood supply through the common carotid artery. The red dye used for the injection of the arteries is the usual substance employed for this purpose, namely, alkanin. This material in various dilutions does not afford a sufficient contrast for the study and comparison of the small excretory ducts and the capillaries. The gross relationship is more clearly indicated when two colors are used. The difficulty seems to be due to the necessity of macerating the injected specimens in concentrated hydrochloric acid, and subsequently washing away the débris. In this manner, the injected masses are freed from the overlying hard and soft tissues. A search of the literature failed to find a description of any blue dye which would withstand the action of the concentrated hydrochloric acid. The color of alkanin is not changed during digestion, but apparently no blue dye has been described with properties identical in this respect to the red dye.

A combination of dyes may be used, however, which, although temporarily discolored by concentrated hydrochloric acid, regains a distinct hue when washed in water. Such dyes are, for example, crystal violet and brilliant green. A 10 per cent. solution of both of these dyes is made in acetone and used for coloring the celluloid preparations. Subsequent digestion of the injected material by means of concentrated hydrochloric acid decolorizes the dye temporarily. When digestion is complete and the injected mass washed with water, the color returns.

The great advantage of these dyes is apparent in experimental work requiring simultaneous injection of different systems or of different structures.

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* From the George Williams Hooper Foundation for Medical Research, University of California Medical School.

Reprinted from The Journal of the American Medical Association
Jan. 20, 1923, Vol. 80, p. 181

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Reprinted from TRANSACTIONS OF THE AMERICAN ASSOCIATION OF
GENITO-URINARY SURGEONS, VOL. XV, 1922

RENAL COUNTERBALANCE[1]

AN EXPERIMENTAL AND CLINICAL STUDY WITH REFERENCE TO THE SIGNIFICANCE OF DISUSE ATROPHY

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I. INTRODUCTION

Modern writers accept too readily the occurrence of compensatory hypertrophy of the kidney. Evidently its observation has been so long general as no longer to attract notice or comment. At any rate its true significance and importance, particularly in relation to the surgery of the kidney, has been almost entirely overlooked. Twenty years ago the subject was kept very much alive by the many wordy battles of the cellular pathologists, with some creditable experimental work, as to renal hypertrophy versus renal regeneration. Apparently the complete victory of the former has left the subject flat and without interest. The remarkable advance in renal diagnosis and renal surgery since then calls for a revival of interest and study, but with an entirely different viewpoint.

The problem of the choice of the better or best operative procedure in the treatment of many renal conditions is still difficult in spite of improvement in the methods of diagnosis and in surgical technic. Mistakes are by no means as frequent or at all comparable to those made before the common use of the cystoscope and ureteral catheterization. But occasionally any surgeon practising in the field of the urinary tract will meet with an unlooked-for result, both in the way of success as well as of failure, and in the case of failure will wish when it is too late that in place of being conservative he had been radical, or vice versa. Some of these surprises can be attributed to technical errors, some to compensatory hypertrophy, but many of them cannot be so easily explained. It is in this latter group that a reconsideration of renal compensation is particularly pertinent.

It is the purpose of this paper to present a study of some experimental and clinical examples of renal compensation with this surgical aspect particularly in mind. Heretofore renal reserve and compensatory hypertrophy have been the sole factors recognized. We shall endeavor to show that renal competition and disuse atrophy are factors that must be reckoned with in the counterbalance that follows many surgical procedures on the kidney, and renal disease without surgery as well.

II. HISTORICAL REVIEW

A. Observation of compensatory hypertrophy

Rayer (1) in his remarkable book gives, as early as 1841, as good a clinical description of compensatory hypertrophy as has appeared. Various aspects of the subject have been since discussed, notably by Albarran (2, 3, 4), Anzilotti (5), Bizzozero (6), Carnot (7, 8), Cohnheim (9), Eckardt (10), Fiori (11, 12), Galeotti (13), Ghoreyeb (14), Morel and Verliac (15), Peruzzi (16, 17), Pisenti (18), Pousson (19, 20), Podwyssozki (21), Ribbert (22, 23, 24, 25, 26, 27), Simon (28), Sacerdotti (29, 30), Stoerk (31), Tuffier (33, 34), Virchow (35), and Wossidlo (36). Four types of renal hypertrophy have been observed:

1. *Unilateral or mass hypertrophy.* The hypertrophy of a healthy kidney in compensation for the loss of function on account of disease in the opposite side is a well known fact. The kidney is often remarkably increased in size but preserves its form and normal appearance (Rayer, 1). Some interesting observations have been made with respect to the pathological process leading to destruction of the other kidney. In the renal destruction by hydronephrotic atrophy, following a complete ureteral block, hypertrophy of the opposite kidney is perfect and comparable to that which follows experimental nephrectomy (Albarran,

2, 3). In aseptic lithiasis the hypertrophy may be slight and the enlarged kidney often presents sclerotic zones resulting from a "diasthenic nephritis." With such diseases as pyonephrosis, renal tuberculosis and cancer there may be no compensatory hypertrophy of the good kidney on account of an inhibition (Albarran, 2). It cannot therefore be said that hypertrophy is always proportional to the amount of kidney destruction.

The recognition of hypertrophy of the remaining kidney after nephrectomy has been universal and was known long before nephrectomy was a surgical practice, from the results of experimental production in animals. The experimental work of Simon (28) on 30 dogs before he undertook to do the first nephrectomy on man is classical. This hypertrophy, however, is not a complete anatomical compensation or substitution, as will be shown later (paragraph B).

2. Circumscribed hypertrophy. The hypertrophy of isolated areas in a diseased kidney has been frequently observed. Destructive processes, with a few exceptions of diffuse lesions, do not affect the whole renal parenchyma simultaneously. The unaffected areas may show considerable hypertrophy (Rayer, 1). There is group injury with group compensation. The parts of the kidney spared by the disease take the place of those which succumb (Albarran, 2). Trauma, tumor, infarction, infectious nephritis, stone, retention, may all distribute their action unevenly in the different renal lobes of the same kidney.

In studying chemical nephritis many investigators have found also that all parts of the kidney do not participate to the same degree and that after a time compensatory hypertrophy of the more healthy parts occurs (Chauffard, 37; Golgi, 38; Nauwerck, 39, 40). Charcot and Gombault (41) describe islands of injury and islands of hypertrophy in nephritis, and many histologic studies of small granular kidneys (Köster, 42; Weber, 43; Ziegler, 44), of interstitial nephritis (Fortlage, 45; Golgi, 38; Ribbert, 22, 27), and of parenchymatous nephritis (Golgi, 38; Albarran, 2) point to a definite attempt to shunt the renal function to a small, less injured portion of the renal parenchyma. Thorel (46) describes compensatory hypertrophy of uninjured renal tissue in cases of infarction, which Albarran (2) finds in every way analogous to that seen in partial resections. Ribbert (27) noted in the kidney of a syphilitic infant areas atrophied by interstitial nephritis and areas showing compensatory hypertrophy.

3. Bilateral hypertrophy. The increased functional activity in very chronic severe diabetes has been supposed to lead to an hypertrophy of both kidneys (Rayer, 1; Albarran, 2; Pousson, 19; Sacerdotti, 29). Fletcher, in Osler's Modern Medicine, says they are often enlarged but does not attribute this enlargement to hypertrophy. Curiously, degenerative changes affect Henle's loop only. That the kidneys are enlarged and congested in diabetes insipidis has often been observed also. Of much more interest and significance is the fact that Sacerdotti (29) has produced bilateral hypertrophy experimentally by transfusing the blood of several doubly nephrectomized dogs into a second dog previously bled. Emge (47), however, failed to find renal hypertrophy in rabbits fed on large quantities of urea, and Carnot (8) reports 20 cc. of urine fed daily inhibits hypertrophy.

4. Renal anomalies. The enlarged kidneys found with congenital anomalies form an analogy in renal compensation which is more apparent than real. Congenital absence or agenesis of one kidney occurs about once in 2650 cases (Piersol, 48). Secher (49) quotes statistics compiled by Scheur and Sternberg, which show that in 119,055 autopsies collected from the literature by them there was one case of absence of one kidney in every 5953 cases. Scheur and Sternberg themselves presented seven cases of absence of one kidney, collected from their group of 81,500 autopsies. In those reports which give careful measurements it is shown that the solitary kidney equals in size and weight that of two normal kidneys for an individual of similar age and size (Rayer, 1; Abel, 50; Lyon, 51; Moore, 52; Clark, 53; and Radasch, 54; who collected 255 cases in 1908), whereas the compensatory hypertrophy that follows unilateral destruction, nephrectomy for instance, never doubles the renal mass. The solitary kidney is not, therefore, a true analogy. In its embryonic development a normal amount of renal tissue, even though unilateral, has been provided. In the case of the congenitally small or infantile kidney, which is accompanied by a large pardner, the total renal substance again equals that of two normal kidneys. There is hypoplasia on one side and hyperplasia on the other (Gruber and Bing, 55). Apparently in these instances of congenital enlargement, or hyperplasia, the renal reserve has not been used up to the same degree as in the hypertrophy of true compensation. The substitution is embryologic and not compensatory.

A number of curious instances of unilateral hypertrophy of the whole body have come to autopsy. Gordinier (56) finds 28 cases reported in the literature up to 1918. In some of these cases the kidney of the hypertrophied side has been noted as being much larger than its mate (Hutchinson, 57), but in Gordinier's (56) personal case no disparity in the size of the kidneys was found. The difference in the size of such kidneys would presumably be due to circulatory conditions and not to compensatory factors anyway.

B. The time required for the production of hypertrophy

1. As estimated by measurement of the kidney. Most of the estimations of the increase in size of a kidney have followed experimental nephrectomy. Morel and Verliac (15) determined the respective and total weight of the two kidneys in a series of white rats as a control. In another series they performed left nephrectomy and after varying periods of four to fifty days sacrificed the animal and got the weight of the remaining hypertrophied kidney. In the "first phase," four to ten days, the kidney increased 32 to 37 per cent in weight; in the "second phase," ten to twenty days, 9 to 16 per cent; and in the "third phase," twenty to fifty days, 11 to 16 per cent. The early greater enlargement they attributed to hyperemia. Carnot (8) found the opposite kidney of the rabbit enlarged on an average of 33 per cent (16 to 53 per cent) fourteen days after nephrectomy. Paolo Fiori (11) and Tuffier (33) estimated, independently, that twenty to twenty-five days are required for the anatomical completion of a compensatory hypertrophy after nephrectomy, and in this time one-fifth to one-sixth of the original kidney weight is replaced. Carnot (8) attempted to modify the regular course of hypertrophy by physical, chemical and physiological agents. X-ray caused occasionally a considerable increase (an 88 per cent increase in five days in 1 case), an average increase of 50 per cent, or double. Theobromine, lactose and cantharides gave very variable results. Urine in amounts of 20 cc. per day inhibited the course of hypertrophy (0 to 3.8 per cent and 2.4 per cent), but extracts of kidney, fetus, thyroid and hypophysis promoted its development (respectively, 33 to 39 per cent; 55 to 60 per cent; 50 to 53 per cent; 32 per cent, to 44 per cent, and 48 to 61 per cent). Nigst (58) found that a complete enervation of a kidney failed to influence the development of hypertrophy in rabbits.

2. As estimated by study of the renal function. We can find only a few careful studies of functional repair after nephrectomy. The native reserve power of a healthy kidney is generally regarded as sufficient to care for function after nephrectomy. Pilcher (1913) reduced the kidney substance by ligating branches of the renal arteries. He states that loss of one-half renal blood supply, by ligating one branch of both renal arteries, does not cause any noticeable disturbance in renal function as measured by urea nitrogen excretion, which remained practically normal even with reduction of kidney substance to one-fourth. Karsner, Bunker and Grabfield (60) (1915) followed the non-protein nitrogen of the blood after reduction of kidney substance and found a distinct increase lasting for three days only, and that removal of two-thirds of kidney substance did not prevent the excretion of urine in normal amounts and concentration. Rowntree and Geraghty (61) estimated that two to three weeks are required after unilateral nephrectomy for phenolsulphonephthalein to be excreted as normally by two healthy kidneys. By following the fluid and chemical constituents of the urine Fiori (11) estimated that ten days are required for complete restoration of function. André Weill, however, using Ambard's constant, estimated that it took two months in dogs for the remaining kidney to take over the work of two kidneys. He had previously fed his dogs on meat until the urea concentration in the urine had become maximum and constant, so that in his experiments the reserve kidney had to compensate for a distinct overload. Ribbert (26) observed that it took "several days" after nephrectomy for the remaining kidney to be able to excrete indigo-go-carmine in the same time as in normal animals and that in old animals the remaining kidney never did become able to excrete all of the dye stuff in the normal time period. Wossidlo (36), also with the aid of indigo-carmine, finds that about six weeks are required for a return to a normal equilibrium after nephrectomy.

C. Histologic studies of hypertrophy

1. The progressive changes of hypertrophy. In reviewing the literature of this section it is well to keep in mind the complexity of renal structure, a few of the striking instances of which may be profitably mentioned. The blood supply to the kidney is unique. In amount it is truly enormous. In distribution it has no parallel. It may vary in amount nineteen times as much as the average supply to other organs. In their distribution the capillaries terminate in glomeruli under high pressure and then empty into a second capillary bed. And most striking of all, there are apparently few primary nutrient blood vessels in the kidney. Doubling the activity of such an organ must have a tremendous circulatory effect. No less significant in this connection is the fact that full embryonic development of the organ is delayed for from two to six years after birth, as evidenced by the persistence up to this time of embryonic glomeruli (Councilman, 63).

It is not surprising then that the early effect of increased function overwhelms the circulatory system. Seventy-four hours after nephrectomy there is an intense congestion, and even diapedetic infiltration of the interstitial tissues. The epithelium lining glomeruli and tubules swells and the cells multiply by mitosis to the point of filling the tubular lumina and give rise upon desquamation to the formation of cylinders or casts. This process shows group distribution in that it does not reach the same severity in all areas. The acute hyperemia gradually subsides (four to ten days—Morel and Verliac, 15), leaving the glomeruli and tubules in places enormously dilated and fringed often with very large epithelial cells (Golgi, 38; Eckhardt, 10; Rosenstein, 64; Van der Stricht, 65; Albarran, 2; Sacerdotti, 29). The initial change is in every way analogous to an acute interstitial nephritis (Pousson, 19). In the second stage there is a gradual increase in the size of glomeruli and tubules. The epithelium becomes very much hypertrophied, and the convoluted tubular elements particularly are enlarged with respect to the thickness of their walls and the size of their lumina. Glomeruli also may grow to be two to three times their normal volume and are structurally perfect (Albarran, 3). These hypertrophic changes again show first a group distribution, which gradually spreads uniformly throughout the kidney.

2. *Hypertrophy vs. regeneration or hyperplasia.* It will hardly be necessary more than to summarize the literature of this paragraph, even though it forms the bulk of that available on compensatory hypertrophy.

The question of an actual formation of new glomeruli and tubules was under debate for many years. Material used for study was so various as to render conclusions difficult of conciliation. New formation of both glomeruli (Lorenz, 66) and tubules (Ribbert, 23) was observed in young animals, but Councilman (63) states that embryonic elements may persist even in man up to the sixth year. The remarkable mitosis which is present in areas of parenchymatous and interstitial nephritis has led to misinterpretation, many workers believing it a sign of true regeneration. Thus Lipsky (67) observed new formation of epithelium after inflammation, and Albarran (3) explains this finding as nothing more than connective tissue granulation. Simpson (68) studied 81 small kidneys of patients eighteen months to seventy-one years of age and in 5 found fairly large sized fields of pure buds. These new tubules are usually lumenless and contain two to five cells on transverse section. Sprouting of buds from preëxisting tubules was also observed. In these same kidneys elongation and widening of the tubules was noted.

Stoerk (31) had previously described similar conditions in hypernephroma as methods of new formation of renal parenchyma. Before 1900 Petrone (69), Pisenti (18), Kummel (70), Tizzoni and Pisenti (71), Tuffier (34), Vogel (72), and Tillmans (73) had expressed a belief in renal regeneration, but during the same period disbelief in it was voiced by Aschoff (74), Anzilotti (5), Ballowitz (75), Barth (76), Beckmann (77), Beumer (78), Bizzozero (6), Cohnheim (9), Enderlen (79), Eckhardt (10), Falk (80), Fiori (11), Golgi (81, 82), Gudden (32), Guttmann (83), Grawitz and Israel (84), Oliver (85), Köster (42), Lancereaux (86), Leichtenstern (87), Loeb (88), Lorenz (66), Lustig and Galeotti (89), Galeotti and Villa-Santa (13), Maas (90), Wolf (91), Nauwerck (39), Nothnagel (92), Podwyssozki (21), Perl (93), Peruzzi (16, 17), Ribbert (23), Rosenstein (64), Simon (28), Stoss (94), Thorel (46), Valentin (95), and Weigert (96). Since 1900 no one has found true renal hyperplasia. Recently Berti (97) has studied the question from a slightly different angle in man, concluding also that new formation does not occur. As Councilman says, the kidney has a marked capacity for repair but, except in the very young, none for true regeneration of tissue. Epithelial cells may show regeneration of new cells and the compensatory hypertrophy of healthy portions may simulate new formation, but a true regeneration of renal parenchyma does not occur.

3. Repair in hydronephrosis. The anatomical and functional restoration of a hydronephrotic kidney following relief of the ureteral obstruction is most interesting. It was in the course of an experimental study of repair in hydronephrosis in white rats that the relationship of repair to counterbalance, which has been the incentive for the present contribution, was first appreciated. Work in experimental hydronephrosis (Johnson, 98; Donati, 99, 100; Hinman, 101) shows that a kidney which has had a complete obstruction of its ureter for two weeks or less may not only regain normal function but be capable also of undergoing a complete compensatory hypertrophy after opposite nephrectomy. We were further able to show with white rats (Jour. Urol., 1919, 174) that "the effect of relief of back pressure on hydronephrosis of twenty-one to sixty days is shrinking in size with preservation of kidney shape and the grouping of all tubular remnants in one relatively large median sagittal lobule;" that "the tubular and glomerular elements of the central lobule show definite hypertrophic changes;" that "in hydronephrosis of ninety-five days or longer no such central lobule is noticeable;" that "the central lobule developed after relief of obstruction in hydronephrosis up to sixty days shows many hypertrophic convoluted tubules which stain intensely by the intravital method;" that "new formation of tubules cannot be demonstrated;" that "the recovery and hypertrophy of already markedly atrophic structures and their renewed susceptibility, or even hypersusceptibility, to intravital staining is evidence of the ability of hydronephrotic kidneys up to certain stages (sixty days) to undergo considerable anatomic and functional restoration;" and that "in hydronephrosis of ninety-five days or longer recovery, either anatomic or functional, of the secretory elements cannot be demonstrated." We registered our impression in this same article (pp. 150, 153 and 154) of the unfairness of these experiments, as well as those of Johnson (98), Donati (99, 100) and others, to the hydronephrotic kidney; in the one instance by requiring it immediately to do double duty by removal of the opposite kidney, and in the other (pp. 152 and 153) by placing it in unequal competition with the thoroughly capable compensatory kidney when nephrectomy is not performed.

The clinical importance of the above experimental facts is quite evident and the two following statements rob it of none of its significance: "Plastic operations on a large hydronephrosis of a capacity of more than 5 or 6 ounces is usually unsuccessful," (Braasch, 102), and "The possibility of restoration of function upon relief of obstruction is long past when the kidney can be palpated. The damage produced in a fully developed hydronephrosis is permanant." (Walker, 103.) No doubt both Drs. Braasch and Walker in making the above statements presume that the opposite kidney is perfectly healthy.

D. The amount of renal parenchyma necessary for life

The same general conclusion that about one-third of the combined weight of two kidneys is required to maintain life has been independently reached by many different experimental investigators. De Paoli (104), in 1892, by resection of part of a kidney and nephrectomy on the other side, found that an animal could live on one-half of one kidney. Paolo Fiori (11) (1901) by similar experiments estimated the amount of kidney substance necessary to support life as one-twelfth to one-fifteenth per kilo of the body weight of the animal. Castaigne (105) (1902) determined that one-third to one-fourth of the combined kidney weight is necessary for life. Vitzou (106) obtained a similar result. Mauchle (107) (1894) had reached the same general result after nephrectomy on one side and ligature of several of the smaller branches of the opposite renal artery; and Pilcher (59) (1913) reduced the kidney substance also by ligature of branches of the renal artery to one-fourth and found that the urine remained normal in quantity. Tuffier (33) found 1.5 grams of kidney substance per kilo sufficient to maintain life, and found an increase in weight by hypertrophy of that remaining to 100 per cent, which Broca (108), however, claims is no argument against regeneration and accuses Tuffier of a mathematical error. Rose-Bradford (109) noted death in one to six weeks after removal of three-fourths of the renal substance, and this finding has been confirmed by Bainbridge and Beddard (110). Pearce (111), by allowing compensatory hypertrophy to occur after the removal of half of a kidney, was then able to remove still more, sometimes as much as three-fourths of the total kidney substance in dogs, without change in the nitrogen metabolism; and he found that after the second resection there was an increase in the hypertrophy of the secretory elements over the hypertrophy already produced by the first resection. More recently Karsner, Bunker and Grabfield (60) (1915) have been able to reduce the renal mass by two-thirds without affecting the normal amount and concentration of urine excretion. Schwarz (112) comes to the conclusion that there is a relative proportion between the excretory power and the mass of excreting parenchyma. However, Bonardi (113) concludes from an experimental study that nephrectomy notably diminishes the animal's resistance to infection and poisons; and Giordano (114) and Ollive and Le-Meigneu (115) even go so far as to estimate the diminution in working value of a man with one kidney as 50 per cent.

E. The question of biologic changes due to nephrotoxins

Lindemann (116) (1900) claimed that he could produce a substance in the serum of guinea pigs specifically toxic for the kidney of a rabbit by injecting an emulsion of rabbit's kidney into guinea pigs; that this "nephrotoxin" in the serum of the guinea pig would not only produce albuminuria but also death by uremia in three to five days after intravenous injection in the rabbit, and that the rabbit's kidneys presented necrosis of the epithelium of the convoluted tubules resembling that from renal poisons. This finding was confirmed by Nefedieff (117) (1901), Bernard and Meissner (118), and Castaigne and Rathery (105, 119, 120) (1902), the latter of whom have most vigorously championed the idea. Paolo Fiori (11, 12) (1903) noted a difference in the histologic appearance of the opposite kidney after ureteral ligature and after nephrectomy and attributed the more pronounced trophic alterations in the case of ligature as due to abnormal products in the circulation. Amos (121) (1905) reports the unbelievable finding of death in rabbits in every instance after simple ureteral ligature under conditions suggesting uremic coma, and attributes death as due "presumably" to the absorption of toxic substances still being secreted by the ligatured kidney. Hirsch and Mascke (122) (1912) allowed a nephrectomized dog to become uremic when its carotid was connected to the jugular of a healthy dog, the kidneys of which as a result showed on examination marked changes in their epithelium. Albarran and Bernard (4) (1903), Pearce (123) (1904) and many others have not been able to confirm these findings and deny the existence of specific nephrotoxins, cytotoxins, etc. The experimental work of Rosenow, while not directly related to the kidney, renders attractive the idea of the specific nature of tissue proteins. This subject is by no means satisfactorily closed.

F. Renal atrophy

The small infantile kidney, already referred to (II, *A*, 4) is not an atrophied kidney. The parenchyma of such a kidney and the products of its activity are normal. They are abnormal quantitatively only. Geraghty and Plaggemeyer (124) (1913) found 3 of this type among 36 cases of renal atrophy in 3940 autopsies, and they pointed out their true diagnostic and surgical importance. Simpson (68) (1915) classified contracted kidney into five groups:

(1) Genuine or primarily contracted kidney.

(2) Athero-sclerotic kidney (patchy sclerosis—Zeigler).

(3) Secondary contracted kidney (chronic interstitial nephritis).

(4) Hydronephrotic contracted kidney (Aschoff).

(5) Senile kidney.

All of these forms are retrogressive metamorphoses and when bilateral may be explained as the result of degenerations (albuminous, fatty, hyalin and amyloid) or necroses, due to the destructive action of bacterial or mineral toxins or the products of disordered metabolism. Occasionally nephritis is unilateral, and several writers (Babouneix, Braasch) have drawn attention to the atrophic condition of many of these unilateral types. In line with the main argument of this paper it occurs to the writer that this atrophy is largely the result of, or is, at least, hastened in its progress by, diminution or loss of function on account of the increased ability of the opposite healthy kidney. In all of Braasch's 28 cases of "atrophic pyelonephritis" total function was normal indicating that the opposite kidney had undergone complete compensatory hypertrophy.

Pathologists (Adami, 125) "recognize that the two prime factors which tell upon the health of the individual cells are nutrition and the performance of function." Excess as well as lack of nourishment, or excess as well as lack of function, may lead to atrophy. Disuse atrophy is of common knowledge for many organs and structure, but so far as our search of the literature goes we can find not a single reference to disuse atrophy of the kidney. Nutritional atrophy as we understand it for other organs is also difficult of application to the kidney, because, with the exception of a few to the pelvis and capsule, the kidney probably possesses no true nutrient arteries. Nutrition and function in the kidney are almost synonymous. Lack of function means poor nourishment, and a rich or excess of nourishment is not possible without an increase of function. The work of a kidney is therefore an important factor for either hypertrophy or atrophy.

G. Summary of historical review

The body has normally a relative amount of renal tissue. A large individual will have large kidneys and a small individual, small kidneys. If in development one kidney only is formed it will equal the weight of what two normal kidneys would. If in development one large and one small (infantile) kidney are formed the two together will equal the weight of two normal ones. If there is destruction of renal tissue by disease, trauma or operation, that part remaining uninjured or the least injured will attempt to take over the lost function. For this counterbalance nature has provided a certain reserve, but this native reserve is quite limited and unable to carry for any length of time any sort of an added burden. Renal tissue meets this demand, if at all continued, by growth, by compensatory hypertrophy. According to the nature and portions of injury this compensatory growth may be a unilateral mass hypertrophy or a circumscribed group hypertrophy. It requires a period of time for its completion and is roughly proportional to the functional demand. Doubling the demand never results in doubling of the renal mass by hypertrophy. But the demand may be so great as to overwhelm the tissue and an actual atrophy from excess of function results, whereas, if the demand is gradually increased a much greater burden eventually will easily be borne. Renal reserve therefore is potential and not actual and is measured by the degree of hypertrophy that is possible. Regeneration of renal tissue does not occur in response to an increased functional demand except in the very young. The degree of hypertrophy possible is very great. One-third (one-fourth under conditions of gradual reduction) of the normal renal mass may so hypertrophy as to counterbalance the loss of the other two-thirds (or three-quarters). There is no mention in the literature of renal atrophy from loss of function as a factor in renal counterbalance.

III. EXPERIMENTAL STUDY[2]

A. Introduction

The problem of renal counterbalance is complicated and made more interesting by the division of the renal mass into two equal and separate portions. This anatomical partition has been long regarded by surgeons as a blessing but may often prove to be misleading. It establishes an obvious competitive basis for renal compensation. Injury more frequently affects one portion, and competition between this and the remainder is thus rendered unequal. We may take economics of industry in point of illustration. The renal mass and renal function may be said to represent an equilibrium respectively of supply and demand. The demand, or the total renal work required, shows general variations only, such as occur with the growth of an individual and the normal fluctuations of excretion following changes in diet and exercise. But the supply, or the total renal tissue that performs the required work, may show marked alterations. Usually these changes are those of loss or insufficiency as a result of disease, trauma or operation. But occasionally the change may result in a surplus of renal mass or supply, as, for example, after the removal of ureteral stones or after repair operations in hydronephrosis. The process of a reëstablishment of an equilibrium of supply and demand that follows these alterations in the supply or demand is the process of renal counterbalance. Injury from disease, trauma, etc., or surgery are responsible for the anatomical alteration by which tissues are thrown out of balance, and the establishment of a final anatomical counterbalance may take years for its completion, even though compensation of function is effected within a few days. In the establishment of such an anatomical counterbalance we believe that, first, compensatory hypertrophy as a result of the action of increased functional activity upon renal reserve, and, second, disuse atrophy as a result of loss or diminished functional activity through an unequal competition with the above are important factors.

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[2] The writer wishes to express his appreciation and great indebtedness for the generous assistance in many of the following animal experiments of Dr. A. E. Belt, fellow in Hooper Research Foundation 1919–1922, and Resident Urologist of the University Hospital, 1921–1922. No less commendable has been his zealous interest in searching the medical literature, and whatever of completeness there is in the bibliography is due largely to his invaluable assistance.

One can think of numerous animal experiments to test the correctness of these statements regarding renal counterbalance. Such experiments were begun by us in 1916. The results have all been confirmed by at least three, and in some cases eight, repetitions. Some of the experiments require a period of about two years for their completion, and this being realized beforehand, three to four animals were run together. After the completion of the initial experiment the result was often so striking as to lead to the necessity of a control experiment of like duration.

The relationship of reserve power and of competition to renal compensation will appear more logically if dealt with separately.

B. Renal reserve and compensatory hypertrophy

1. As observed anatomically. The simplest experiment that will test renal counterbalance is nephrectomy. A healthy kidney is thus suddenly burdened with just double the work to which it is accustomed. We wish to express our indebtedness to Dr. Thomas Addis for permission to mention his very interesting, unpublished results in this connection. He finds differences in the degree of hypertrophy of different portions of the renal tubule. In rabbits, one to three months after nephrectomy, the glomeruli have increased 20 per cent in size over those of the removed kidney, the collecting tubules 7 per cent, while the proximal convoluted tubules have increased 60 per cent. The average total increase was 70 per cent. Complete ligature of one ureter accomplishes as effectively as nephrectomy the development of a compensatory hypertrophy. We have been able to study over 400 hypertrophic rat kidneys after complete ligature of the opposite ureter for periods of one to six hundred or more days' duration. The hypertrophic changes as measured by an increase in the size of the kidney were complete on an average of twenty to thirty days. There was no demonstrable increase in size beyond this period, even of those kidneys doing double duty for almost two years. In no case was the size of the kidney doubled nor did the increase ever even approach to this degree. In figure 1 is shown a series of hypertrophic rat kidneys typical of the different periods, and in table 1 the measurements are summarized. It is seen that the average total increase is 20 per cent of the normal.

TABLE 1

Measurements of hypertrophied right kidneys in rats after total ligation of left ureter

DURATION OF HYPERTROPHY	AVERAGE KIDNEY MEASUREMENTS			NUMBER OF RATS REPRESENTED	AVERAGE WEIGHT OF RATS
	Length	Width	Depth		
	cm.	*cm.*	*cm.*		*gms.*
None; normal	1.63	0.90	1.01	30	194
4 days	1.63	0.90	1.03	7	192
8 days	1.87	0.93	1.03	10	196
14 days	1.90	0.96	1.05	21	201
28 days	1.90	0.92	1.09	20	193
42 days	1.95	0.99	1.15	17	197
56 days	2.00	0.97	1.12	14	195

Hypertrophic kidneys of rabbits, cats, dogs and pigs after opposite ureteral ligature have also been studied, but in relatively small series. The findings correspond to those for rats. Doubling of renal tissue by hypertrophy has never been observed.

The histologic changes of compensatory hypertrophy vary with the period of examination. There are three distinct stages: First, the initial congestion, second, the active mitosis and growth, and third, the final equilibrium of hypertrophy. It is difficult to understand how there can be increased functional activity during the initial hyperemia that overwhelms a kidney with the sudden demand for more work. The picture closely resembles an acute inflammation with diapedesis, swelling and leukocytic infiltration. That there is an increase of function shows how dependent renal activity is upon blood volume, which must be enormously increased. During the second stage the pronounced karyiokinesis of cells is apparently an attempt to repair the acute damage and to meet the new demand by hypertrophy. The complete hypertrophy of the third stage is proportional in a way to the demand. Hypertrophy is a relative term and a cell may grow slightly or double or treble its original size, and apparently the size it attains is dependent up to a certain limit upon the degree and progressive character of the strain to which it is subjected. In figure 2 is shown a micro-

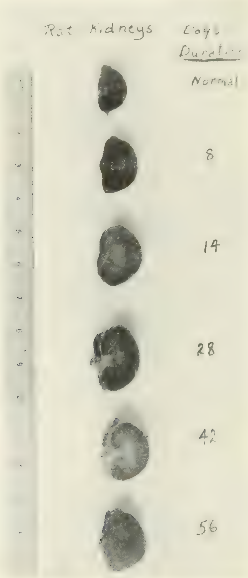

FIG. 1. Photograph (¾ size) of the hypertrophic kidney of rats of similar weight and age at varying periods after opposite complete ureteral ligature. The kidneys of twenty-eight, forty-two and fifty-six days are practically all of the same size.

photograph of a normal rat kidney stained intravitam with diamin blue, and in figure 3 a hydronephrotic repair hypertrophy. The difference in the size of these convoluted tubules is quite obvious. Both rats weighed approximately the same, 200 grams.

2. *As observed functionally.* The usual clinical supposition is that the native reserve power of a healthy kidney is sufficient to do double the amount of work on demand. The number of individuals who have weathered nephrectomy with no functional disturbance of any kind is its warranty. So far as ordinary tests go, such as the amount and concentration of the urine, blood nitrogen and phenolsulphonephthalein output, the remaining kidney is just about equal to the task. But if the least overload is put on this kidney its insufficiency is at once apparent.

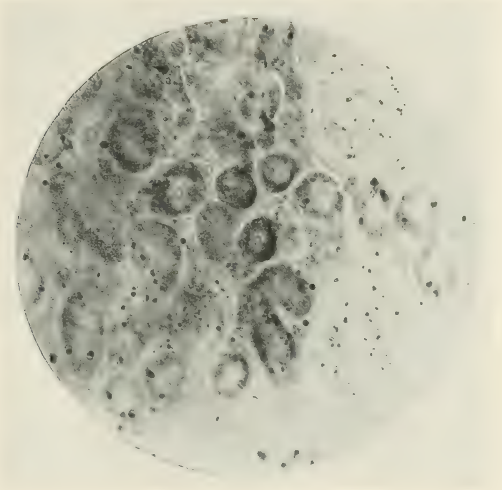

FIG. 2. Microphotograph (high power) of normal rat kidney stained intravitally. The convoluted tubules only take the stain. Compare with figure 5, a hypertrophic rat kidney similarly stained and magnified.

Kutzmann,[3] in collaboration, has made carefully controlled and definite experiments in dogs to test this point. He finds that in the first two to three days after nephrectomy the excretion of urea and phthalein are definitely diminished and there is a corresponding rise of urea and non-protein nitrogen in the blood. A graphic chart of his findings is reproduced in figure 4. Phthalein is normal by the third or fourth day but urea excretion not until about the tenth or eleventh day. Putting the kidney under a greater strain by injecting urea prolongs the period of unbalance. A control experiment with dogs under ether for one hour, but

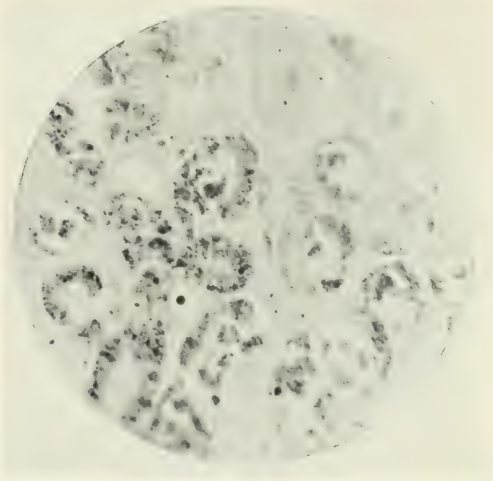

FIG. 3. Microphotograph (high power) of a hypertrophic repair nodule (middle specimen of figure 5) stained intravitally, which shows more intense staining and greater hypertrophy even than that of a complete compensatory hypertrophy, and a remarkable hypertrophy as compared to the normal of figure 2, magnified similarly.

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[3] This work has not been completed for publication and I wish to thank Dr. H. A. R. Kutzmann, surgical interne, University Hospital for his courtesy in allowing me to publish this part of it.

without operation, gave no similar alteration in the phthalein or in the blood and urine nitrogen. Goldschmidt and Pearce have shown that splenectomy has no effect on renal function, so that it seems fair to rule out shock from operation or anesthesia as a contributing factor; and a just conclusion is that the native reserve of a kidney is insufficient to take care of as much as 50 per cent increase in work, and that this additional demand through increase of function leads to hypertrophy.

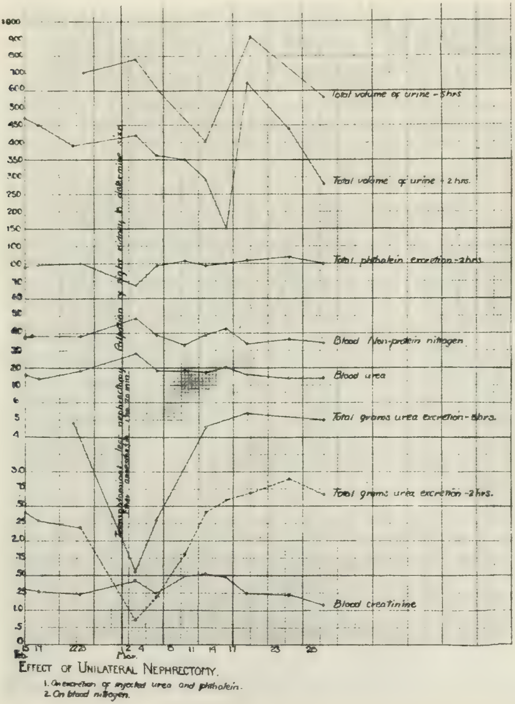

FIG. 4. The rise in blood nitrogen and fall in nitrogen and phthalein secretion shows definite incapacity of the reserve of one kidney to care for the loss of function of a kidney during the first few days after nephrectomy.

C. Renal competition and disuse atrophy

It has been shown that in the case of normal kidneys upon the removal of one the remaining kidney is at first unable to do the work of both and that it becomes competent to do so only after it has developed an hypertrophy. The questions naturally arise: When such an unilateral hypertrophy is complete, will it form a more or less independent renal system? Will it prove to be self-sufficient? Has it acquired a monopoly of renal function, and because of this can it now crowd off the market all inferior competitors? By surgical measures we can place different renal masses in competition and test such a law of survival of the fittest. It is understood that by competition we refer to quality of work and not quantity, and by unequal competition we do not refer to an unequal amount of work but to its relative ease and efficiency. An infantile kidney, for example, does much less work than its partner, but so long as it works efficiently it holds its own in competition. A repair hydronephrosis also does less work but inefficiently, and what will be the result when placed in competition with a perfectly capable hypertrophic mate?

Another experimental example other than repair hydronephrosis of disuse atrophy, which is rather unusual and bizarre and difficult of certain explanation, will follow transplantation of one ureter into the duodenum. The circulation of such a kidney has not been disturbed and it continues to get blood of the same quality as its fellow, but on account of a complete reabsorption of its products of activity its work is completely wasted and lost. In so far as a help to the opposite kidney is concerned, it might as well be completely removed, and the opposite kidney will undergo just as complete compensatory hypertrophy as if it had. But why should not the duodenal-transplant kidney likewise undergo a complete compensatory hypertrophy? And if it does, will it continue indefinitely to do double duty uselessly? These two sets of experiments will be presented separately.

1. Experimental hydronephrosis. The competitive factor in renal counterbalance and the subsequent disuse atrophy of renal tissue can be illustrated by comparison of the results that follow four types of experiments with hydronephrosis:

a. The early result of ureterocystoneostomy in hydronephrosis, when the opposite kidney is undisturbed.

b. The late result of ureterocystoneostomy in hydronephrosis, when the opposite kidney is undisturbed.

c. The result of ureterocystoneostomy in hydronephrosis, with opposite nephrectomy.

d. The result of ureterocystoneostomy in hydronephrosis, when the work of the opposite kidney is gradually destroyed by a partial ureteral obstruction.

a. The early repair in hydronephrosis upon relief of ureteral obstruction by transplantation into the bladder. In the repair experiments on white rats, even with sixty days' complete obstruction, it was found that considerable repair occurred after relief of the obstruction by hydroureteral transplantation. This repair was evidenced by the formation of a secretory nodule, which was proportionally greater in the short periods of obstruction than in the long periods, but the repair period in all of these experiments was only from thirty to sixty days; that is, it was within thirty to sixty days of the ureterocystoneostomy that the rat was sacrificed (fig. 5). The two points in question are: Would these repair nodules appear differently if the period of competition with the compensatory kidney on the

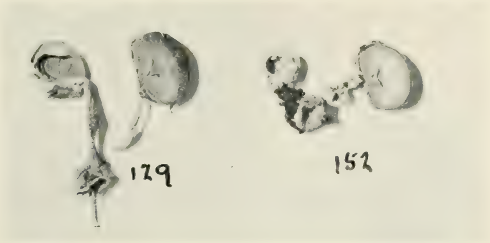

Fig. 5. Photograph (¾ size) of two hydronephrotic repair kidneys with their respective hypertrophic mates. There is a median-sagittal or central repair nodule present in each. A microphotograph of the first specimen, sixty-day complete ureteral obstruction with one hundred and twenty-nine-day repair period after ureterocystoneostomy, is shown in figure 3.

opposite side were prolonged, and would they also appear differently if the competition were equalized by a concurrent injury on the compensatory side?

The following two protocols are of dogs with left ureteral obstruction of forty-four and sixty days and a repair period of unequal competition with the opposite hypertrophic kidney of forty-three and fifty-five days, respectively, and are reported in full in order to show the destructive result of severe renal infection. The experimental results are often difficult of interpretation because of the complicating action of infection or imperfect urinary drainage, and we wish the reader fully to understand our appreciation of this difficulty.

Protocol 1

Ureteral obstruction and repair period each of forty-four days. Long-haired sky terrier, male, no. 17-63.

November 14, 1917. Weight 15¾ pounds. Left ureter divided near the bladder between silk ligatures, under ether. Convalescence and recovery good.

December 28, 1917. Weight 16 pounds. Ether narcosis; surgical asepsis; left hydroureter exposed, divided just above ligature; incomplete emptying of sac by compression of 38 cc. of dirty, brownish fluid, showing microscopically enormous numbers of blood and pus cells. Hydroureter transplanted to bladder.

January 7, 1918. Convalescence has been good. Dog active. Phthalein 60 per cent in two hours.

February 9, 1918. Weight 16½ pounds. Ether, right ureter exposed, doubly ligated near the bladder and divided between the ligatures.

February 11, 1918. Death; acute hemorrhagic nephritis of right kidney with localized peritonitis. There had probably been bilateral infection so that upon ligature of the right ureter the hypertrophied kidney was unequal to the additional strain. Blood vessels at infected foci ruptured and a marked retro-peritoneal and intracapsular hemorrhage resulted, with extravasation of urine and spread of infection to the peritoneum. The pelvis of the left kidney is contracted. The minor calyces are formed of thickened fibrous bands and the atrophic cortex shows diffuse pyelonephritis.

Protocol 2

Ureteral obstruction of sixty days, repair period of fifty-five days. Long-haired grey dog, no. 17-66, weight 22 pounds.

November 16, 1917. Ether; double ligature and division of left ureter near bladder.

December 28, 1917. Slight attack of distemper.

January 16, 1918. Weight 23 pounds. Left ureter transplanted to bladder. By compression 117 cc. of dark, dirty fluid aspirated.

February 26, 1918. Weight 23½ pounds. Right ureter transplanted to duodenum. 1 cc. phthalein intravenously; none obtained through bladder.

February 27, 1918. Bladder; faint trace of phthalein in two hours; 40 cc. urine.

March 13, 1918. Dog has been very sick with distemper. Phthalein, faint trace in two hours. 210 cc. of slightly cloudy, watery urine.

March 15, 1918. Death from distemper. Duodenal transplant in healthy condition. Right ureter is patent but some obstruction is present, as evidenced by slight ureteral dilatation. Duodenum is not inflamed. Left kidney is shrunken, very blue and flabby. The left ureteral transplant to the bladder is in very good condition and the bladder is filled with light cloudy fluid. Upon section the kidney is seen to have two well developed repair zones of renal parenchyma, one at either pole. The remainder is a thick structureless sac wall with radiating fibrous bands carrying blood vessels (fig. 6-2).

Comment. The above two experiments illustrate the destructive action upon the renal parenchyma of a pronounced infection and are reported in order to dispel any probable inference of our failure to recognize infection and injury as important factors in producing a late atrophy. We have been able to show, however, that in spite of infection or injury from back pressure, renal parenchyma placed under different circumstances does not atrophy (see types c and d), and on the other hand, that while they may hasten it, neither can be regarded as the sole factor in producing some types of what may be looked upon as examples of disuse atrophy.

b. The late changes that follow relief of obstruction in hydronephrosis where the opposite compensatory kidney is healthy and undisturbed. We have tested the above question of competition by keeping our animals for long periods after ureterocystoneostomy before sacrificing them. In all of the animals in which a competition had been active for one year or longer a very considerable to total atrophy of the hydronephrotic repair kidney is found. The forty-five-day hydronephrosis of dog 17–56 after four hundred forty-three days' competition has completely atrophied (fig. 6, *3*), in spite of an increased demand during the last sixty-nine days on account of an imperfect uretero-rectal transplant of the compensatory kidney. Dog 17–13 (fig. 6, *1*) is interesting from the standpoint of repair after partial obstruction. At the end of six months of partial left ureteral obstruction by means of a rubber band, when the left pelvic capacity was 895 cc., the large tortuous hydroureter was transplanted into the bladder; seven months later there is one small hypertrophic repair nodule at the lower pole of a remarkably shrunken kidney. Microscopic section shows persistence of functionating renal radicals in the nodule and the presence still of atrophic glomeruli in the rim where all tubular elements are missing. The following 4 protocols are examples of late atrophy.

Protocol 3

Ureteral obstruction of thirty days, repair period of two hundred and ninety-seven days. 48-pound dog, no. 17-179.

June 28, 1917. Ligature and division of left ureter.

July 28, 1917. Left hydroureter transplanted to the bladder.

November 17, 1917. Total phthalein, 83 per cent.

May 28, 1918. Right nephrectomy. Blood urea, 18.64 mgm. per 100 cc.

May 30, 1918. No phthalein in one hour, ten minutes.

June 1, 1918. Blood urea, 86.7 mgm. per 100 cc.

June 5, 1918. Blood urea, 354.2 mgm. per 100 cc. Dog not eating; listless. Temperature 34.6°. Sacrificed. Left kidney is small and atrophic but with two zones of repair tissue at either pole similar to the kidney in 17-66. But these repair nodules were unequal to the task of total renal function after the removal of the completely hypertrophied right kidney and the dog would have died in a very short time of uremia. This experiment is of interest and most significant when compared to the final result in dog 20, protocol 8, in which

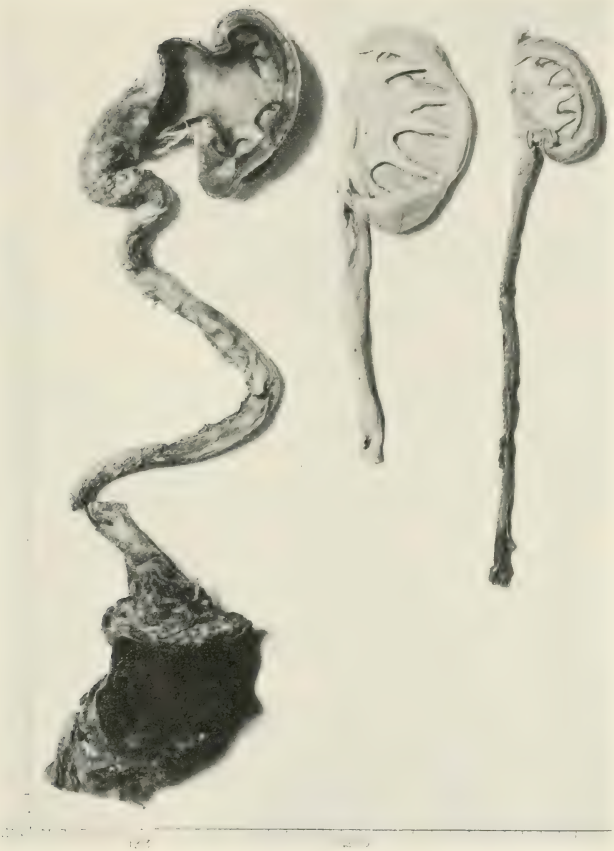

FIG. 6. Photograph (about $\frac{3}{4}$ size) of three hydronephrotic repair kidneys in dogs 17-13, 17-66 and 17-56. The first (17-13), with a capacity of 5 cc., is a seven months' repair after six months, partial obstruction, at which time the hydronephrotic sac held 895 cc. There is one repair nodule at the lower pole. The second (17-66) is a fifty-five-day repair after sixty days complete obstruction. There is a repair nodule at each pole. The third (17-56) is only a forty-five-day hydronephrosis but with three hundred and ninety days' competition with its hypertrophic mate, and shows complete atrophy.

a thirty-day complete ureteral obstruction was found capable of performing total renal work.

Protocol 4

Ureteral obstruction of forty-five days, repair period of three hundred and ninety days. No. 17-56, old grey dog, weight 21¾ pounds.

March 6, 1917. Double ligature and division of left ureter.

April 30, 1917. Left hydroureter transplanted to the bladder.

November 17, 1917. Total phthalein, 65 per cent.

May 7, 1917. Total phthalein, 50 per cent.

May 9, 1917. Right ureter transplanted to the rectum. Blood urea, 27.1 mgm. per 100 cc.

May 14, 1917. Dog sick. Weight 22 pounds. Blood urea, 156.2 mgm. per 100 cc. Phthalein output through the bowel was a very faint trace.

May 17, 1918. Blood urea, 60.6 mgm. per 100 cc. Dog somewhat improved but sacrificed. At autopsy a pronounced wound infection with localized peritonitis in region of the rectal transplant is found. The right kidney shows an acute pyelonephritis with beginning hydronephrosis. The left kidney (fig. 6-3) is a little atrophic structure with kidney shape and a small freely patent ureter but no secreting parenchyma.

Protocol 5

Ureteral obstruction one hundred and eight days, repair period four hundred and eleven days. Brown mongrel dog, male, no. 17-64.

November 14, 1917. Weight 24¼ pounds. Double ligature and division of left ureter.

March 2, 1918. Left ureter transplanted to bladder. Ureter markedly dilated (1.8 cm.). Undergoes peristalsis while lying beneath peritoneum and before being disturbed. After division of its end 200 cc. of clear amber colored fluid was emptied by its own peristalsis, and then an additional 249 cc. by compression.

November 17, 1918. Phthalein, 83 per cent.

May 7, 1919. Weight, 27 pounds.

May 14, 1919. Transplanted right ureter to rectum. Local peritonitis and death May 18, 1919. The left is a small shrunken remnant of a kidney, about which evidences of a previous pronounced collateral circulation are seen. The ureter is freely patent and there is no evidence of urinary back pressure. The kidney shows complete atrophy.

Protocol 6

Partial ureteral obstruction of six months, repair period of seven months. Large brown dog, weight 33½ pounds, no. 17-13.

August 11, 1917. Rubber band placed about lower end of left ureter so as partially to obstruct its lumen.

February 12, 1918. Weight 22½ pounds. Rubber band removed and the ureter transplanted to the bladder to insure relief of the obstruction. The ureter was very markedly dilated (1.5 cm.) and tortuous and was seen to undergo active peristalsis, during which it would shrink to about 0.8 cm. in diameter, or one-half. Upon division it emptied by its own muscular activity a little over 750 cc. of fairly clear fluid, and it was possible by compressing the hydronephrotic sac to empty 145 cc. more—total, 895 cc. The hydroureter was then transplanted to the bladder.

February 16, 1918. Dog has developed a pronounced cystitis. Urotropin given by stomach tube.

February 19, 1918. Active and well.

August 7, 1918. Weight, 35 pounds, 8 ounces. Total phthalein, 61 per cent.

September 6, 1918. Right ureter transplanted into rectum. Left kidney not palpable and left ureter about 0.6 cm. in diameter, healthy looking but tortuous and under going active peristalsis.

September 11, 1918. Death. Ureter transplant into bowel had slipped and urine pouring into peritoneal cavity, producing generalized peritonitis. The left kidney is markedly shrunken in consideration of its previous pelvic capacity of over 895 cc. and shows one single repair nodule at its lower pole (fig. 6-1), which, however, has actively functionating radicles.

Comment. The above 4 experiments illustrate the pronounced to total atrophy following hydronephrotic injury, infection and functional disuse. That hydronephrotic atrophy and destruction secondary to infection are not the sole factors in the process is shown by the results in the two following types of experiments, in which a distinct demand or need for preservation of renal parenchyma—even though it is injured—is experimentally produced at a time when the injured tissues can respond to it, and what would otherwise have atrophied from diminishing activity now hypertrophies from increased activity.

c. The repair of hydronephrosis following ureterocystoneostomy plus opposite nephrectomy. This experiment puts a most difficult test upon renal tissue. A kidney which has suffered a complete ureteral obstruction for a period is suddenly relieved by ureterocystoneostomy, and at the same time the other kidney, which has been doing all the renal work for this period, is at once removed. The remaining renal tissue, injured by the prolonged and complete obstruction, is immediately called on, not only to recover its lost function but to double it. It is remarkable that a kidney whose ureter has been completely occluded even for two weeks can show as much reserve and repair ability as to regain not only its lost function but also compensate for the loss of the opposite kidney. We have performed several times successfully such an experiment but have never been able to have an animal live after opposite nephrectomy if the obstruction was prolonged on the hydronephrotic side beyond fourteen days (protocol 7). Aside from its interest as an example of renal reserve and compensatory hypertrophy the result has its greatest significance in connection with experiment type d, in which a kidney completely obstructed for four weeks, or twice as long, recovered a complete compensation for the loss of the opposite kidney, but under distinctly different circumstances.

The first kidney at the left in figure 7 is from a cat on which there was a complete obstruction of the left ureter for twelve days and a period of repair after ureterocystoneostomy of ten days, when the right kidney was removed. The cat lived in good health for another one hundred ten days, when it was killed (protocol 7).

Protocol 7

Left ureteral obstruction of twelve days, repair period of ten days, then right nephrectomy. Sacrificed one hundred and ten days later. Large grey cat, no. 17-1.

August 3, 1917. Double ligature and division of left ureter. Loose carbolic ligature placed about right.

August 9, 1917. Total phthalein, 22 per cent.

August 15, 1917. Left ureter transplanted to bladder. Both ureters are dilated. Though the right is patent, it is more dilated than the completely obstructed left.

August 19, 1917. Total phthalein, 45 per cent.

August 25, 1917. Right nephrectomy.

September 6, 1917. Total phthalein, 35 per cent.

November 16, 1917. Cat well and active. Total phthalein, 50 per cent.

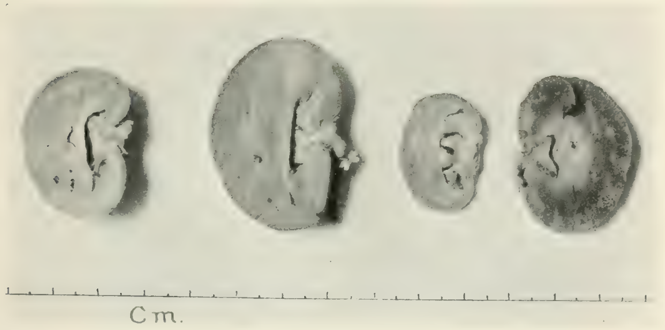

FIG. 7. Photograph of 4 cat kidneys. (1) Kidney of a cat (17-1, protocol) in which a twelve days' complete ureteral obstruction was followed by ureterocystoneostomy and opposite nephrectomy. There is complete repair. This kidney has undergone in addition a complete compensatory hypertrophy. The cat was perfectly well and had normal total function 120 days later, when sacrificed. Microphotograph of this kidney is shown in figure 20. Cat's weight, 6 pounds. (2) Kidney with a fourteen-day ureteral obstruction and a twenty-day repair of a cat weighing $8\frac{1}{2}$ pounds. (3) Repair hydronephrosis of a cat weighing $3\frac{3}{4}$ pounds. Sixty days complete ureteral obstruction and one hundred days before sacrifice after ureterocystoneostomy. (4) Hypertrophic mate of 3.

December 14, 1917. Cat sacrificed on account of a severe diarrhea. The left kidney is slightly enlarged, appears normal macroscopically and shows compensatory hypertrophy microscopically (figs. 8 and 9). There is no pelvic residual or dilatation (fig. 7-1).

d. The late changes that follow in the repair of a hydronephrosis after ureterocystoneostomy when a partial obstruction is placed on the opposite ureter. In order to show that the atrophy and disappearance in the course of one to two years (type b, experiment) of what was at an earlier period of one to three months of repair a well preserved and respectably repaired hypertrophic nodule, (type a. experiment) in a hydronephrosis relieved of its obstruction, are due more to loss of function and disuse atrophy in consequence of an unequal competition with the increased function and compensatory hypertrophy of the opposite healthy kidney, than to progressive destruction from degeneration and disease, one can equalize competition by a gradual injury of the compensatory side. The degenerative changes instituted by the primary hydronephrosis remain unaltered, so that if they are responsible for the final atrophy in one experiment they ought to produce atrophy just the same in the other. Variations in the local resistance to the action of infection with functional activity and

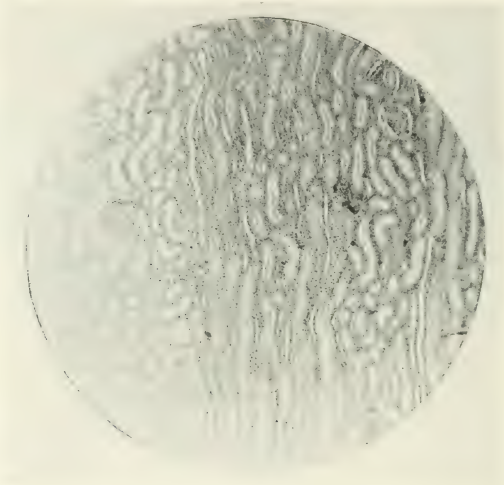

Fig. 8. (Cat 17-1, protocol 7.) Microphotograph (low power) of the twelve-day hydronephrotic repair as well as complete compensatory hypertrophic kidney shown in figure 7-1. The tubules seem to be lengthened and more tortuous, as well as broader. Glomeruli are also hypertrophied.

inactivity are also probabilities worthy of mention in considering renal atrophy from disuse, and constitute a problem for experimental study.

A gradual injury of the complete compensatory kidney can be produced simply by a partial obstruction of its ureter with a rubber band. In a year's time there will be practically a complete hydronephrotic atrophy of its previous hypertrophic tissues (protocol 6). To all intents and purposes it is a dead kidney. The animal will now have no secreting renal tissue, provided the degeneration proceeded on the hydronephrotic repair side, (as it did in type b, experiment), and death from renal insufficiency would result. But the gradual increase infunctional demand so

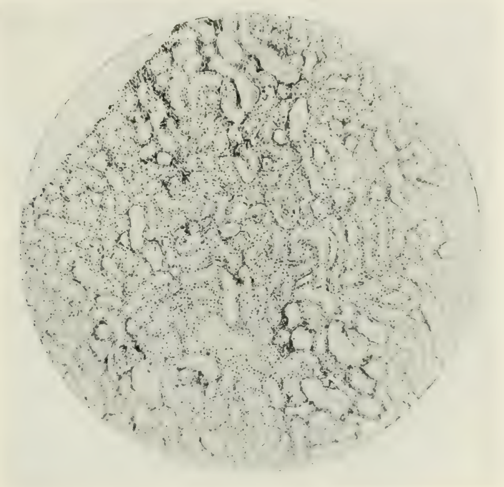

Fig. 9. (Cat 17-1, protocol 7.) Microphotograph (low power) of another area of the repair kidney shown in figure 7-1. The injury produced by the twelve-day ureteral obstruction is manifest by the persistence of dilated and destroyed tubules intermixed with hypertrophied and healthy tubules. Two atrophic glomeruli are seen in the section.

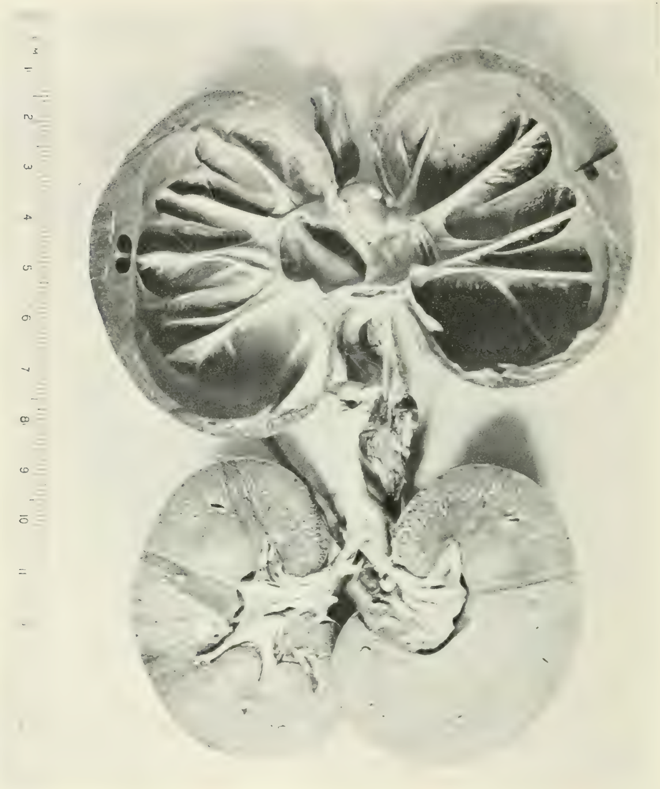

FIG. 10. (Dog 22-39.) Photograph of a simple hydronephrosis of thirty days complete ureteral obstruction and the opposite hypertrophic kidney. Hydronephrotic atrophy is quite pronounced. The arterial systems of kidneys of forty-one days' hydronephrosis injected with $BaSO_4$ are shown in the next figure (figures 11 and 12).

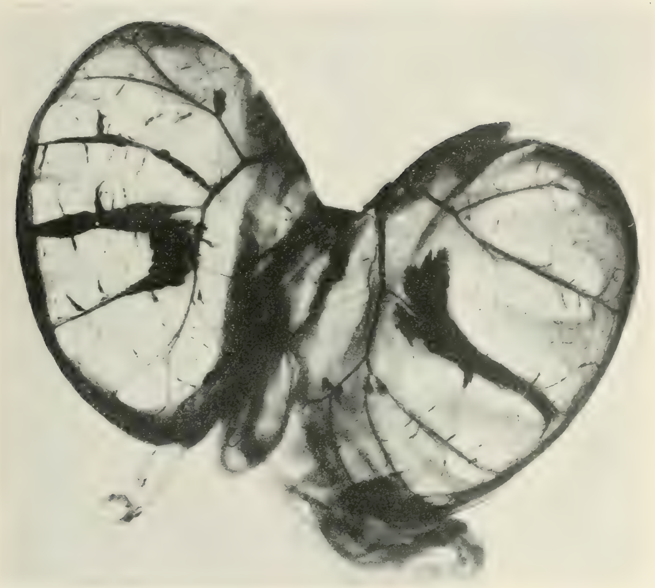

FIG. 11. (Dog 22-12.) Photograph of roentgenogram of barium sulphate arterial injection of a forty-one-day hydronephrosis in which the dog was sacrificed and barium sulphate solution injected at once after death. The attenuation of the circulatory tree of the hydronephrotic kidney is remarkable and is particularly significant when compared to the reproduced arterial tree of the kidney as shown in figure 12, which, although a forty-two-day hydronephrosis, had had two days' relief from back pressure, due to ureteral obstruction.

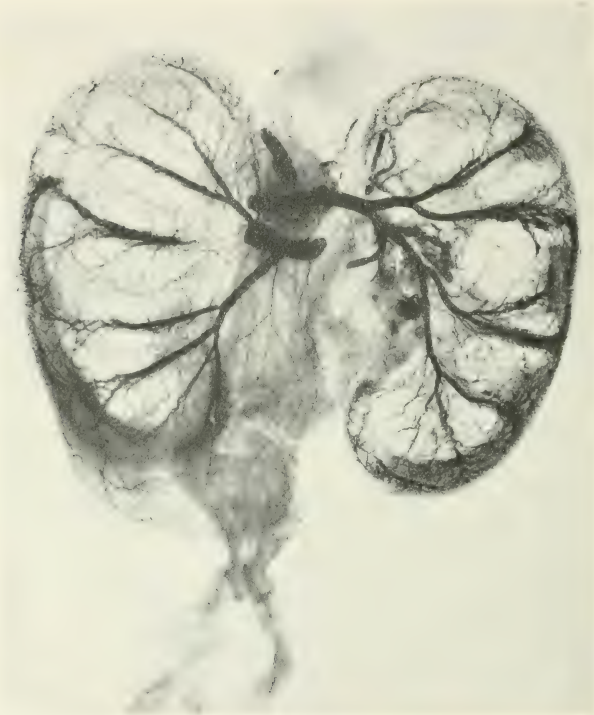

FIG. 12. (Dog 22-36.) Photograph of roentgenogram of barium sulphate arterial injection of a forty-two-day hydronephrosis but in which the dog was sacrificed two days after the ureteral obstruction had been relieved. The same solution of $BaSO_4$ was used under equal pressures for both preparations shown in figures. Yet there is a remarkable revascularization apparent in the kidney relieved of the arterial back pressure, and this remarkable increase in blood supply must have been almost immediately after the removal of the intrapelvic pressure. It is significant of repair possibilities and of great interest in connection with the result in dog 21-39, figures 13 and 14.

progressively stimulates an otherwise hopelessly injured kidney or so sufficiently raises its resistance to the injurious action of infections, toxins, etc., that it regains not only its lost ability but so hypertrophies that it now easily and successfully carries the burden of total renal work (protocol 8). Figure 10 is a photograph of a simple thirty-day hydronephrosis in a dog and is presented for comparison. It is seen that there is already quite a considerable degree of hydronephrotic atrophy. In figure 16 is shown a kidney which had had two days longer complete obstruction, and yet it now shows no evidence, either in gross or microscopically, of hydronephrotic atrophy. The demand for activity has been transferred from the right compensatory side to the repair side, which has responded to this need in a truly wonderful manner. It is a remarkable experimental example of a final anatomical readjustment in renal counterbalance.

Changes in the circulation that follow relief of ureteral obstruction are beautifully shown in figures 11 and 12 (protocols 10 and 11) and are significant of the dependence of renal function on blood supply. This amount of revascularization allows one to understand the remarkable anatomical readjustment in type d, experiment, the following protocol of which is given in detail:

Protocol 8

Dog 21-39. Shepherd, male, weight 42 pounds.

November 1, 1920. Ligation of left ureter. Blood: Urea nitrogen, 18 mgm. per 100 cc.; non-protein nitrogen, 34 mgm. per 100 cc. Phenolsulphonephthalein, 70 per cent in two hours. The abdomen is opened in a midline incision under the usual ether anaesthesia and aseptic precautions. The incision extends from the symphysis pubis upward about 4 cm. The bladder is seized with the fingers and drawn gently upward into the wound. A double silk sature is passed beneath the left ureter just above its junction with the bladder and with it the ureter is firmly and doubly ligated. The ureter is severed between these two ligatures and the stumps are dropped back into the wound. The abdominal wound is closed with no. 2 chromic sutures. During the next week the dog is seen to make an uneventful recovery.

December 2, 1920. *Left ureterocystoneostomy.* Under ether anesthesia and aseptic precautions the abdomen is opened in the midline.

The bladder is lifted up into the wound and the large tortuous left hydroureter is seen. It is approximately 1 cm. in diameter and filled tightly with urine. The end of the ureter is carefully freed from its bed and about 100 cc. of slightly smoky urine is drained into a large test tube when the ligated tip is cut off. A no. 00 catgut suture is then passed through the adventitia of the ureter, which is slit open for about 0.5 cm. on the side opposite to the suture, the free ends of which are threaded into small milliner's needles. A short incision is then made through the bladder wall in its posterior aspect just a little lateral to the midline and the threaded milliner's needles passed through this and out through the bladder wall about 1 cm. above their point of entry, where they appear with a 3 mm. space between them. Traction on the catgut draws the ureter through the bladder wall and when the ends of the fine catgut are tied the ureter is held with its slit-end open within the bladder. The ureter is more firmly fixed to the bladder wall externally with fine interrupted stay sutures, which pierce the adventitia of the ureter and the external coats of the bladder. The abdominal wound is closed with no. 2 chromic sutures. Recovery is prompt and without incident.

December 9, 1920. *Partial ligation of right ureter.* Under ether anesthesia and surgical asepsis the abdomen is opened in the midline just below the umbilicus. The peritoneum is smooth and glistening. There are few adhesions in the region of the bladder vault. The right ureter is identified in its bed as low down as possible without disturbing the recent operative area behind the bladder. A short length of ureter is freed in an area about 3 cm. from its point of entry into the bladder and a rubber band 2 mm. wide is placed around it and stretched. The ends of the band thus stretched are tied with silk so as to form a moderate constriction of the ureter. The wound is closed with no. 2 chromic. The animal recovered slowly from the operation, eating little for four or five days. The wound healed readily, however. A period in which his condition seemed improved then followed, lasting four to five days. The dog again refused food for a second period of five or six days and lay very still in his cage. Following this he slowly grew better. A month after operation he seemed quite normal and ate normal amounts.

November 29, 1921. Blood from jugular for nitrogen partition: Urea nitrogen, 26 mgm. per 100 cc.; non-protein nitrogen, 49.0 mgm. per 100 cc.; creatinine, 1.93 mgm. per 100 cc.

January 4, 1922. *Sacrifice. Autopsy.* The dog is in excellent condition; weight 42.5 pounds. Coat is long and glossy. Has been passing well formed stools and clear urine. Phenolsulphonephthalein first hour, 53 per cent; second hour, 20 per cent. Blood: Urea nitrogen, 21 mgm. per 100 cc.; non-protein nitrogen, 41 mgm. per 100 cc. Killed with ether. There are three operative scars on the abdominal wall, one on each side and parallel to the penis and one in the midline above the penis. The wound areas are all tightly closed and uninfected. There are some scant adhesions to the under surface of each of the abdominal wounds. The mesentery only is adherent here. The abdominal organs, except for the genito-urinary tract, present no lesions whatever. Heart is not enlarged, shows no pathological alterations. Lungs clear. A sample of urine from the right ureter is clear and shows a considerable amount of pigment. The urine from the left ureter is cloudy. It shows an equal amount of pigment. The urinary tract is shown in the reproduced photograph (fig. 13).

Kidneys. The left kidney is normal in shape and slightly enlarged. It collapses readily at the hilus with pressure, showing a moderate degree of hydronephrosis. The ureter of this kidney is very large, being 1 cm. in diameter. It is not tortuous. Its opening lies in the midline of the bladder posteriorly and is fully patent. It readily admits a no. 8 F. catheter. This kidney shows a heavy venous network over its surface external to the capsule. Microscopic section shown in figure 14. The right kidney is very large, has a very thin wall. There is a crescentic nodule at its lower pole 1.5 cm. thick and 7 cm. long, a microphotograph of which is reproduced in figure 15. In all other areas the wall is of paper thinness. The ureter is slightly larger than that of the opposite side. It is 1.3 cm. in diameter. It is much thinner than the left ureter and very tortuous. Near the bladder it presents an obstructed area, at which point the rubber band placed December 9, 1920, is localized. Beyond this it is of normal diameter. The right uretero-vesical orifice through which this ureter opens is normal in all respects and readily admits a no. 4 F. catheter. Gentle pressure on the hydronephrotic sac causes a flow of fluid through this ureteral orifice. The wall of the bladder is normal in appearance. The urethra presents no constrictions nor abnormalities. The respective sizes and weights of the two kidneys were as follows:

	LENGTH (POLE TO POLE)	WIDTH (SIDE TO SIDE)	DEPTH (HILUS TO GREATER CURVATURE)	WEIGHT	PELVIC CAPACITY
	cm.	*cm.*	*cm.*	*grams*	*cc.*
Left kidney....	6.95	2.5	3.0	47	5
Right kidney...	16.0	8.0	7.5	52	400

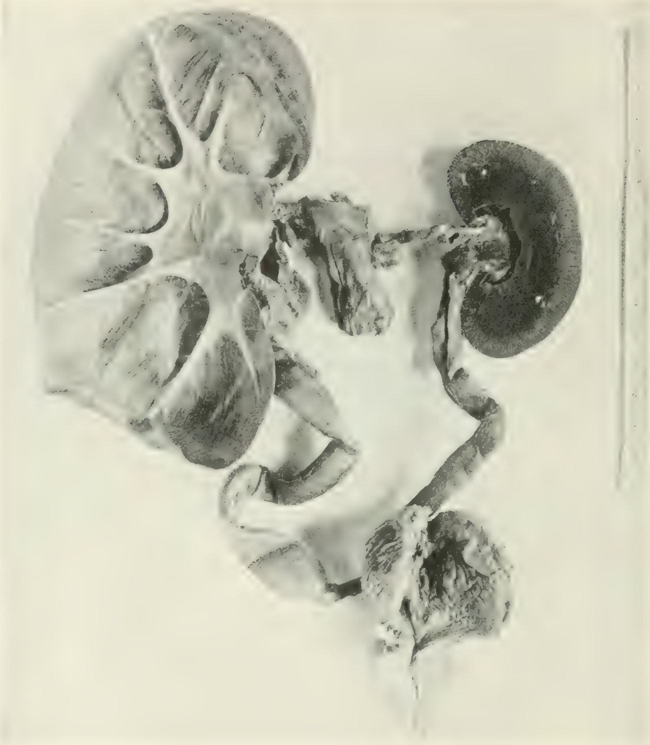

FIG. 13. (Dog 21-39, protocol 8.) Photograph of a thirty-one-day hydronephrotic repair days after ureterocystoneostomy and partial obstruction of the opposite ureter. The freely patent opening of the hydroureter into the bladder is marked by an end of a white match stick. This ureter has not shrunken and is almost as large as the opposite hydroureter belonging to an almost complete hydronephrotic atrophy. This kidney therefore must have been after its thirty-one-day complete obstruction markedly hydronephrotic and in every way analogous in size and condition to the simple thirty-day hydronephrosis shown in figure 10. The difference between them is quite evident after section. The thirty-one-day hydronephrosis has shrunken considerably but preserved its kidney shape and undergone a remarkable repair. There has been a complete anatomical counterbalance, now the previously dead kidney is doing normal and double function and the previously compensatory side has undergone complete hydronephrotic atrophy.

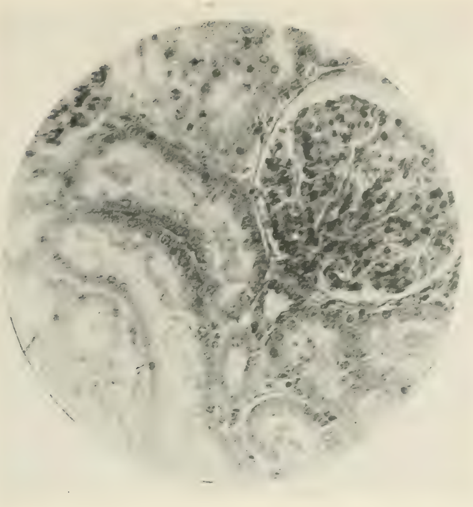

FIG. 14. (Dog 21-39, protocol 8.) Microphotograph of the left hydronephrotic repair kidney of figure 13. Hypertrophy of tubules and glomeruli is evident. Dilatation of tubules is seen to persist in areas, but there is little evidence of a previous thirty-one-day hydronephrosis.

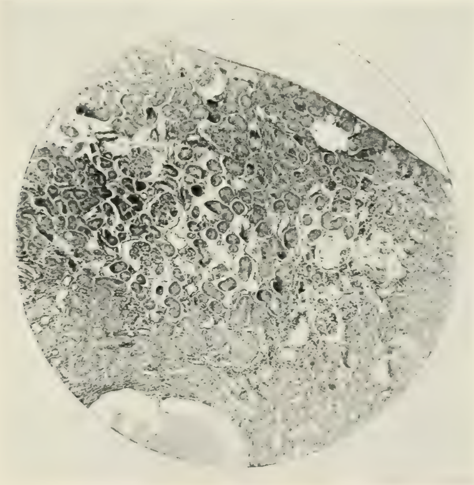

FIG. 15. (Dog 21-39, protocol 8.) Microphotograph of an area in the remnant of parenchyma left at the upper pole of the large hydronephrotic right kidney of three hundred and ninety days partial ureteral obstruction. Glomeruli persist the longest; tubules have almost disappeared.

The nitrogen content of the urine from the left kidney is: Urea nitrogen, 0.0045 gram in 1 cc.; non-protein nitrogen, 0.0065 gram in 1 cc.

The nitrogen content of the urine from the right kidney is: Urea nitrogen, 0.0039 gram in 1 cc.; non-protein nitrogen, 0.0068 gram in 1 cc.

e. Control experiments with simple ureteral transplantation without previous hydronephrosis. There may be readers who feel that the late atrophy of experiment type b is nothing more than the result of the disturbance following ureteral transplantation and that transplantation of a ureter any place produces enough injury to lead to late atrophy provided the other kidney is healthy. The experiment just cited would seem sufficient proof to the contrary. An imperfect ureteral transplant leads by back pressure to renal injury, and the final atrophy in the above counterbalance experiments is no doubt hastened according to the degree of this injury; but the fact that by placing a need for this tissue delays or prevents atrophy (type c), whereas the lack of such need hastens it, indicates that in the latter case lowered function or disuse is an active factor in the late atrophy. The atrophy of a thirty-day hydronephrosis in view of its being opposed to an hypertrophied mate completely capable of doing all the work will obviously be faster than a kidney equal at the start with its unhypertrophied fellow when conditions are altered by ureteral transplantation. If the transplantation is at all nearly successful such a kidney will never atrophy. There are many instances in the literature of vesical transplantation in which the kidney on the transplanted side has remained healthy for years. The only cause for late atrophy when both kidneys are equal at the time of the transplant is injury from ureteral obstruction or from infection, either of which creates an unequal competition from a qualitative standpoint. Coffey has had dogs survive for many months after unilateral uretero-rectal transplantation, and the kidney on this side found to be normal and equal to its mate, and Carrel has had dogs survive for two and one-half years after kidney excision, perfusion and then inversion in Locke's solution for 2 hours when it was transplantated back in place and the ureter sutured together and opposite nephrectomy two weeks later and finds the animal in good health and the kidney normal in appearance after this long period. Perhaps his result would have been quite different if the good kidney had remained undisturbed. This question of the injury to a kidney in consequence of ureteral transplantation is of significance in the interpretation of the results of the following experiments.

2. Ureteroduodenostomy. When a ureter has been successfully transplanted into the duodenum the kidney will continue to function but its work is as completely nullified, so far as urinary excretion is concerned, as by nephrectomy, since there is complete urinary reabsorption.[4] An analogous result would follow the transplantation of a ureter into a vein, an experiment actually performed by Reid. What happens to the transplanted kidney? Will it hypertrophy or will it atrophy?

Protocol 9

Dog 22-39. Thirty-day hydronephrosis; sacrifice. For comparison with protocol 8, Dog 21-39. Little black male mongrel, weight, 19 pounds. Condition excellent.

February 16, 1922. Blood urea nitrogen, 18 mgm. per 100 cc.; non-protein nitrogen, 30 mgm. per 100 cc.

February 17, 1922. Left ureter ligated near the bladder.

March 18, 1922. Blood urea nitrogen, 18 mgm. per 100 cc.; non-protein nitrogen, 34 mgm. per 100 cc. Sacrificed; ether narcosis.

Autopsy. The animal is in excellent condition. There is a midline scar over the lower abdomen. Heart and lungs normal. Abdomen free from adhesions. All organs appear normal. Genito-urinary tract: moderately enlarged kidney on right, normal in appearance. The left kidney is very large and consists of a fluid-filled sac having a capacity of 130 cc. and with only a small amount of parenchyma remaining. The ureter is large and follows a slightly tortuous course to the bladder. It is firmly ligated just above the point of entry into the bladder. The fluid cannot be forced beyond this point from above downward. The bladder and urethra are normal (fig. 10).

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[4] This subject has been more fully dealt with in collaboration with Dr. A. E. Belt in a paper upon ureteral transplantation presented before the Urological Section of the A. M. A., May 22, 1922, at St. Louis.

Kidney measurements

	WEIGHT	LENGTH	WIDTH	DEPTH
	grams	*cm.*	*cm.*	*cm.*
Left kidney.....	37 (empty)	6.52	3.85	4.32
Right kidney....	45	5.75	3.86	3.42

Protocol 10

Protocol of forty-one-day hydronephrosis for comparison with dog 22-36. Dog 22-12, White Spitz, young adult, weight, 23 pounds.

November 30, 1921. Under ether anesthesia, left ureter firmly ligated just above bladder.

January 9, 1922. Sacrificed; ether narcosis.

Autopsy. No abnormalities are noted in the thoracic cavity. The abdominal contents are negative except for the presence of a huge hydronephrotic sac in the left kidney region. A large ureter is also present. The right kidney is a little larger but normal in appearance. Bladder and urethra normal. $BaSO_4$ injection shown in figure 11.

Kidney measurements

	WEIGHT	LENGTH	WIDTH	DEPTH	PELVIC CAPACITY
	grams	*cm.*	*cm.*	*cm.*	*cc.*
Left kidney....	40 (empty)	8.6	5.8	5.15	160
Right kidney...	31	5.7	2.25	3.3	2

Protocol 11

Dog 22-36. Young adult, male, Airedale. Distemper eight months previously, followed by an intermittent twitch of the voluntary muscles, which still remains. Weight, 25 pounds. The dog is somewhat underweight. General condition: good.

February 14, 1922. Left ureter ligated at uretero-vesical junction. Midline incision; ether anesthesia.

March 29, 1922. Under ether anesthesia the left ureter is opened through a midline incision and 185 cc. of a smoky urine is removed from the sac through a no. 8 F. soft rubber catheter, which was run through the dilated ureter to the kidney pelvis. The catheter is carefully manipulated and gentle suction applied until no further contents can be removed. The ureter is again ligated and the wound closed. An analysis of this material shows that it contains a great number of blood cells. There are very few pus cells. The fluid from a centrifuged specimen shows the following contents: Urea nitrogen, 0.0019 gram in 1 cc.; non-protein nitrogen, 0.0056 gram in 1 cc.; chlorides, 0.0068 gram in 1 cc.

March 31, 1922. Blood urea nitrogen, 19 mgm. per 100 cc.; non-protein nitrogen, 36 mgm. per 100 cc. There is no reaction about the wound. Condition excellent. Under ether anesthesia the ureter is again opened and a no. 8 F. soft rubber catheter is introduced to the kidney pelvis; 18 cc. of slightly blood-tinged fluid is removed from the sac. This material contains many red blood cells; no pus. The fluid from a centrifugalized specimen shows: Urea nitrogen, 0.0005 gram in 1 cc.; non-protein nitrogen, unreadable; chlorides, 0.0077 gram in 1 cc.

Ether anesthesia was carried to narcosis and an autopsy performed. The bladder urine shows the following contents: Urea nitrogen, 0.0081 gram in 1 cc.; non-protein nitrogen, 0.0191 gram in 1 cc.

Autopsy. Heart and lungs normal. Abdominal contents show neither abnormalities nor pathological conditions. The right kidney is a little large; capsule seems tight. There is no reaction about it; no adhesions. The left kidney reveals a number of large vessels coursing over its surface. It is approximately the same size as the normal kidney on the opposite side. Its ureter is considerably dilated, though not tortuous. $BaSO_4$ injection of left kidney shown in figure 12.

Kidney measurements

	WEIGHT	LENGTH	WIDTH	DEPTH	PELVIC CAPACITY
	grams	*cm.*	*cm.*	*cm.*	*cc.*
Left kidney....	35 (empty)	7.05	3.05	3.1	20
Right kidney...	58	6.65	3.1	3.3	2

Protocol 12

Right ureteroduodenostomy; left nephrectomy. Death. Dog 138, adult, female, Airedale, weight 29 pounds. Condition excellent.

July 21, 1919. Blood urea nitrogen, 14 mgm. per 100 cc. Phenolsulphonephthalein, 79 per cent in two hours.

July 23, 1919. Right ureteroduodenostomy by H. P. Smith and A. E. Belt. Ether anesthesia, Fowler-Coffey method.

August 8, 1919. Recovery has been uneventful. Blood urea nitrogen, 40 mgm. per 100 cc. Phenolsulphonephthalein, 30 per cent in two hours.

September 18, 1919. Blood urea nitrogen, 60 mgm. per 100 cc. Phenolsulphonephthalein, 70 per cent in two hours. A left nephrectomy is done, lumbar route. Kidney measures: Length 5.5; width, 3.2; and depth, 3.2 cm.

September 20, 1919. Blood urea nitrogen, 72 mgm. per 100 cc.

September 22, 1919. Blood urea nitrogen, 190 mgm. per 100 cc.

September 26, 1919. Blood urea nitrogen, 252 mgm. per 100 cc.;

September 28, 1919. Blood urea nitrogen, 340 mgm. per 100 cc. watery stools. Constantly drinks water; hair sticky, with penetrating odor.

September 29, 1919. 8:00 a.m.: Blood urea nitrogen, 485 mgm. per 100 cc. Catatonic. 5:15 p.m.: Pulse 148 per minute; very weak, eyes glassy, hair sticky, penetrating odor. At 8:10 p.m. a slight rose tinge is noted in the liquid yellow stools which are constantly passing; rigor, with a slight, fine tremor. Pulse rapid, growing irregular. Cheyne-Stokes respiration begins. The eye reflexes disappear. Death at 8:20 p.m.

Autopsy. Rigor mortis marked. Heart and lungs normal. Abdominal contents show normal sheen; intestines in active peristalsis; no adhesions. Right ureter runs without interruption into the duodenum; no kinks; no dilatations. It is about 4 mm. in diameter. The implanted ureter forms a slight dimple on the mucous surface of the gut. Very light pressure on the renal pelvis causes a clear amber fluid to flow through the papilla into the gut lumen. No leakage; no adhesions about the implanted area. Right kidney slightly large but normal in appearance. Pelvic capacity, 7 cc.; slight dilatation.

Kidney measurements

	LENGTH	WIDTH	DEPTH	
	cm.	*cm.*	*cm.*	
Right kidney...	7.0	3.8	3.7	Removed at autopsy, September 29, 1919
Left kidney....	5.5	3.2	3.2	Removed at operation, September 18, 1919

Protocol 13

Right ureteroduodenostomy with left nephrectomy. Sacrifice. Dog 18-14, adult, male, weight 49 pounds. Condition excellent.

October 6, 1918. Blood: Urea nitrogen, 14 mgm. per 100 cc. Phenolsulphonephthalein, 82 per cent in two hours.

October 7, 1918. Right ureteroduodenostomy, ether anesthesia. Fowler-Coffey method.

November 5, 1918. Condition excellent. Wound healed. Blood: Urea nitrogen, 14 mgm. per 100 cc. Bread and milk diet.

November 21, 1918. Left nephrectomy, lumbar route, ether anesthesia.

November 22 to December 3, 1918. Blood: Urea nitrogen steadily rose to 220 mgm. per 100 cc. Dog is dull, somewhat apathetic. Sacrificed.

Autopsy. Slight degree of hydronephrosis and hydroureter. Ostium into duodenum functions, however, and a stream of urine enters duodenum when gentle pressure is applied to pelvis. No leakage, no adhesions. Other tissues appear quite normal.

Protocol 14

Right ureteroduodenostomy; left nephrectomy. Right ureter removed from duodenum and transplanted to skin. Dog 19-25. Large, brown water spaniel, adult, male. Weight 36 pounds.

September 6, 1919. Blood urea nitrogen, 10 mgm. per 100 cc. Phenolsulphonephthalein, 75 per cent in two hours.

September 7, 1919. Right ureteroduodenostomy, Fowler-Coffey method.

September 10, 1919. Blood urea nitrogen, 16 mgm. per 100 cc.

September 14, 1919. Blood urea nitrogen, 19 mgm. per 100 cc.

September 24, 1919. Blood urea nitrogen, 15 mgm. per 100 cc. Phenolsulphonephthalein, 30 per cent in two hours. Left nephrectomy, lumbar route.

September 25, 1919. Blood urea nitrogen, 32 mgm. per 100 cc. From this figure the blood urea nitrogen steadily rose during the next six days.

October 1, 1919. 8:00 a.m.: Blood urea nitrogen, 227 mgm. per 100 cc. Ureter of right kidney brought from duodenum to the skin of the right lumbar region. A short cannula is left in its lumen. Previous to the operation the animal had been dull and apathetic. Shortly after recovery from the ether anesthetic he became very much brighter. 10:00 a.m.: Blood urea nitrogen, 190 mgm. per 100 cc. 4:15 p.m.: Blood urea nitrogen, 122 mgm. per 100 cc. Phenolsulphonephthalein, 21 per cent.

October 2, 1919. Blood urea nitrogen, 48 mgm. per 100 cc.

October 3, 1919. Blood urea nitrogen, 31 mgm. per 100 cc.

October 4, 1919. Blood urea nitrogen, 14 mgm. per 100 cc.

October 5, 1919. Blood urea nitrogen, 17 mgm. per 100 cc.

October 6, 1919. Blood urea nitrogen, 18 mgm. per 100 cc. Weight, 21 pounds. Phenolsulphonephthalein, 19 per cent in two hours. Ureteral opening still draining freely. Killed with ether.

Autopsy. Emaciated, wet. Right ureter draining to right loin. Some pus and granulations on skin surface at this area. Abdomen, smooth, glistening, sheen. Course of ureter to skin is tortuous but no dilatation is noted. Ureter neither distended nor constricted. Pelvic capacity, 10 cc. No adhesions. Heart and lungs normal. Duodenal contents normal; formed stools in rectum.

Protocol 15

Double renal hypertrophy. Right ureteroduodenostomy, nephrectomy, transplantation of right ureter to skin. Dog 20-90, adult, female, doschhund, black, weight, 20 pounds. Condition excellent.

March 3, 1920. Blood: Urea nitrogen, 13.5 mgm. per 100 cc. Phenolsulphonephthalein, 85 per cent in two hours.

March 24, 1920. Right ureter transplanted to duodenum, Fowler-Coffey method. Phenolsulphonephthalein immediately after recovery from operation. First hour, 40 per cent; second hour, 10 per cent; total, 50 per cent.

August 17, 1920. Blood: Urea nitrogen, 17 mgm. per 100 cc.

September 7, 1920. Phenolsulphonephthalein: First hour, 40 per cent; second hour, 5 per cent, total, 45 per cent. Left kidney removed, lumbar route.

September 15, 1920. Blood: Urea nitrogen, 359 mgm. per 100 cc.; creatinine, 2.13 mgm. per 100 cc. Right ureter brought from duodenum to skin. Phenolsulphonephthalein 15 per cent in two hours. Appearance time, twenty-five minutes.

September 16, 1920. Blood: Urea nitrogen, 191 mgm. per 100 cc. Pulse weak, thready. Died early in morning.

Autopsy. Mucous membrane of gums and under surface of the tongue shows multiple ulcerations covered with a greenish, sloughing exudate. The gums are raw and blood-stained. There is a granulating sinus at the site of the nephrectomy done September 7. The sinus does not extend to the peritoneum. Abdominal peritoneum shows no loss of sheen. The ureteral stump into the gut is firmly closed, with no adhesions about it. The projecting papilla on the mucous side of the gut will admit small probe. Left kidney slightly large, pelvis dilated slightly, ureter slightly thickened to two times normal diameter. Lungs show three bluish, gelatinous areas, about 2 to 3 cm. in diameter. Liver and spleen show marked passive congestion.

Kidney measurements

	LENGTH	WIDTH	DEPTH	
	cm.	*cm.*	*cm.*	
Right kidney...	5.35	3.14	3.12	Removed at autopsy, September 16, 1920
Left kidney....	4.55	2.4	2.8	Removed by nephrectomy, September 7, 1920

Protocol 16

Functional study of right and left kidney, with ureteroduodenostomy. Dog 22-16. Brown and white female mongrel, weight 21 pounds.

November 29, 1921. Blood: Urea nitrogen, 24 mgm. per 100 cc.; non-protein nitrogen, 47 mgm. per 100 cc.

January 2, 1922. Blood: Urea nitrogen, 22 mgm. per 100 cc.; non-protein nitrogen, 38 mgm. per 100 cc.

January 14, 1922. Right ureteroduodenostomy, Fowler-Coffey method. The right kidney measures: Length, 4.90 cm., width, 2.45 cm., depth, 2.55 cm.

March 7, 1922. Blood: Urea nitrogen, 16 mgm. per 100 cc.; non-protein nitrogen, 34 mgm. per 100 cc. Phenolsulphonephthalein intravenously: First fifteen minutes, 14 per cent; second fifteen minutes, 12 per cent. The dog is in excellent condition. Given 500 cc. water by mouth; 100 cc. Locke's solution intravenously. Anesthetized with ether, the abdomen is opened in the midline and a catheter is placed in each ureter. The anesthetic is continued while the secreted urine is collected. The resulting urine shows the following concentration and amounts:

	TEN-MINUTE PERIOD FOLLOWING OPERATION			TWENTY-MINUTE PERIOD		
	Amount	Total nitrogen	Color	Amount	Urea nitrogen	Non-protein nitrogen
	cc.	*grams in 1 cc.*		*cc.*	*grams in 1 cc.*	*grams in 1 cc.*
L. K. Urine....................	5	0.0142	Amber	10	0.0056	0.0153
R. K. Urine (ureteroduodenostomy......................	12	0.0061	Light amber	40	0.0042	0.0125

Phenolsulphonephthalein. 2 cc., or 12 mgm., of phenolsulphonephthalein were injected intravenously five minutes after the cannulae were placed in each ureter. The appearance time on the left was four minutes, and on the right, six minutes. Only a faint color of phenolsulphonephthalein was visible in each twenty-minute sample of urine. The concentration was therefore read directly, without diluting, in a Helige colorimeter against a standard made from 2 cc., or 12 mgm., of phenolsulphonephthalein in 1000 cc. of slightly alkaline water. The reading on each side was found to be 5 per cent under these conditions. Since four times as much urine was put out on the transplanted, or right side, it may be stated that four times the amount of phenolsulphonephthalein was excreted by this side. The actual figures, when calculated, are: Left kidney, 0.05 per cent, or 0.006 mgm. in twenty minutes; right kidney, 0.20 per cent, or 0.024 mgm. in twenty minutes. The ether anesthesia was carried to narcosis and an autopsy performed.

Autopsy. (March 7.) Heart and lungs normal. Abdomen: the peritoneum is of normal sheen; no abnormalities noted. The left kidney is a little large but normal in shape. The right kidney is quite large and shows a definite amount of hydronephrosis. Its ureter runs around its lower pole through a mass of loosely formed adhesions and ends in the duodenum. The ureter is dilated definitely. The kidney has a large pelvis, which holds about 12 cc. The parenchyma is encroached upon by the large calyces to a considerable extent. Fluid can readily be forced through the ureter into the duodenum. The other organs are normal.

Kidney measurements

	WEIGHT	LENGTH	WIDTH	DEPTH	PELVIC CAPACITY
	grams	*cm.*	*cm.*	*cm.*	*cc.*
Left kidney....	35 (empty)	6.85	3.3	3.3	20
Right kidney...	32	5.5	2.7	3.1	3

a. The early changes in the two kidneys following transplantation of one ureter into the duodenum. It is possible successfully to transplant a ureter into the duodenum in a high percentage of cases. There is little danger of an ascending infection, which so commonly follows transplantation into the lower gut, and if the technic of Fowler-Coffey is modified so that the ureter is loosely placed, the danger of secondary hydronephrosis may be escaped. A successful transplant therefore will cause little if any injury of the corresponding kidney. That this is true is proved by the direct examination of the kidney within a period of two to four months of the transplant, when there is found no anatomic evidence of inflammation or injury from back pressure upon histologic study of these kidneys. The functional capacity of these kidneys is also established by a number of experiments in which the good left kidney has been removed two to three months after the right uretero-duodenal transplant. In the one group in which nothing further is done the animal invariably dies in nine to ten days, with the same symptoms and signs that follow double nephrectomy (protocols 12 and 13). But in the other group on the eighth or ninth day, when the dog is comatose and shows a blood nitrogen retention of 485 or more milligrams per 100 cc., the right ureter may be transplanted to the skin. The transplanted kidney, instead of frantically pouring large quantities of urine into the duodenum for reabsorption, will now perform actual excretion and will fully recover and renal function be fully restored (protocols 14 and 15). The healthy condition of the right kidney can also be shown after two to three months duodenal transplantation by comparing its function under ether or urethane with the healthy left side, with ureteral catheter in place, as shown in protocol 16.

The left kidney in all of these experiments is burdened with double the work as effectively as if right nephrectomy had been performed. In consequence it undergoes compensatory hypertrophy just as quickly and completely. But the right kidney, as shown in the above paragraph, remains healthy, uninfected and in the cases of successful transplantation, uninjured by back pressure. Its blood supply has not been disturbed. The blood carried through it is of the same character as through the left kidney. Why should it not also hypertrophy? Examination of the two kidneys by sacrifice of the animal at about the second or third-month period does show a bilateral compensatory hypertrophy. The transplanted kidney, which works uselessly so far as urinary excretion goes, responds nevertheless to the same double stimulus in an apparently equally efficient manner as the undisturbed kidney upon which total renal function solely depends. This is well illustrated in figures 16 and 17 and in protocols 15 and 17.

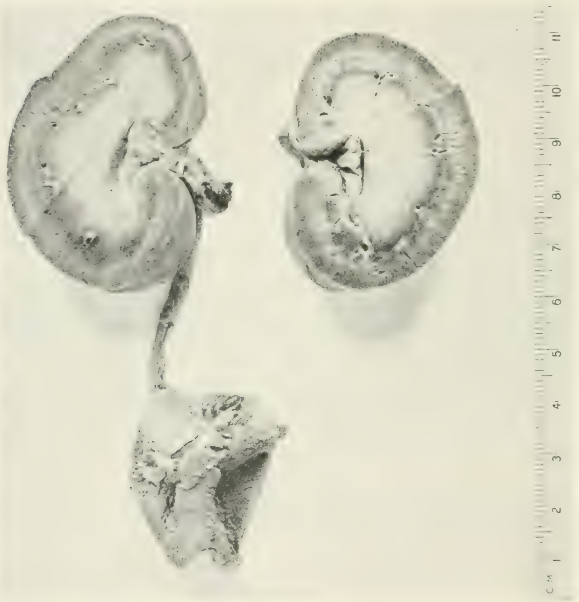


Fig. 16. (Dog 19-139, protocol 13.) Photograph of the two kidneys of bilateral hypertrophy after sagittal section. The hypertrophic condition of the transplanted kidney is apparent and there is no evidence of urinary obstruction, no pelvic nor ureteral dilatation.

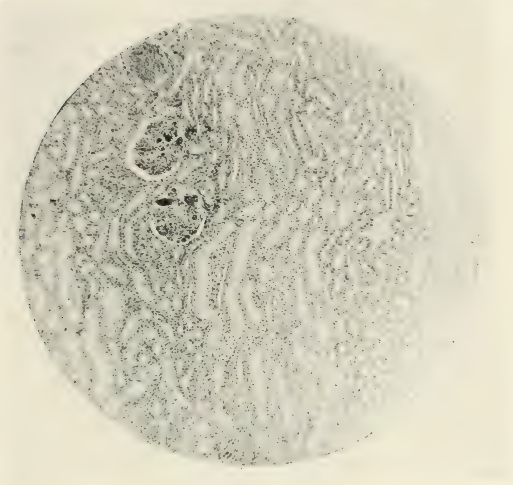


FIG. 17. (Dog 19-139, protocol 13.) Microphotograph of the right kidney of figure 16, whose ureter was transplanted into the duodenum eighty-three days ago. The hypertrophy and healthy preservation of this kidney is clearly demonstrated.

Protocol 17

Double renal hypertrophy. Right ureteroduodenostomy. Killed in eighty-three days. Dog 139, adult, female, black spaniel, weight 19.1 pounds.

July 21, 1919. Blood: Urea nitrogen, 15 mgm. per 100 cc. Phenolsulphonephthalein, 76 per cent in two hours.

July 22 to August 28, 1919. Blood: Urea nitrogen ranged from 15 mgm. per 100 cc. to 13.5 mgm. per 100 cc.—5 determinations.

August 28, 1919. Right ureteroduodenostomy, ether anesthesia.

September 8, 1919. Blood: Urea nitrogen, 19 mgm. per 100 cc.; phenolsulphonephthalein, 75 per cent in two hours.

November 18, 1919. Killed accidentally—stomach tube in lungs.

Autopsy. Lungs filled with frothy, watery fluid. Abdomen: Many adhesions about site of transplantation. Right kidney shows no hydronephrosis; no hydroureter (figs. 16 and 17). Left kidney equal in size.

	LENGTH	WIDTH	DEPTH	
	cm.	*cm.*	*cm.*	
Right kidney..	5.5	2.7	2.6	Removed at autopsy, November 18, 1919
Left kidney....	5.0	2.6	2.7	Removed at autopsy, November 18, 1919

Protocol 18

Right ureteroduodenostomy of four hundred and forty-three days' standing, showing late renal atrophy. Dog 21-35. Female, wire-haired terrier, weight, 24 pounds.

October 22, 1920. Right ureteroduodenostomy, Fowler-Coffey method.

October 29, 1921. Blood urea nitrogen, 19 mgm. per 100 cc.; non-protein nitrogen, 38 mgm. per 100 cc.

January 4, 1922. Blood urea nitrogen, 22 mgm. per 100 cc.; non-protein nitrogen, 43 mgm. per 100 cc.

January 7, 1922. Phenolsulphonephthalein, 43 per cent in one hour. Killed with ether. The phenolsulphonephthalein had been given in the lumbar musculature one hour previous to narcosis. None was found in the duodenal contents at autopsy.

Autopsy. Some omental adhesions to abdominal would; otherwise normal except for atrophy of the right kidney, which is very definitely smaller than the normal. The ureteroduodenostomy is perfect. There is no obstruction visible. The ureteral papilla at the point of transplant stands up clearly in the mucosa. When fluid is injected into the lumen of the ureter above it a large stream passes through the papilla into the duodenum. Heart and lungs normal. No other abnormalities noted.

Kidney measurements

	WEIGHT	LENGTH	WIDTH	DEPTH
	grams	*cm.*	*cm.*	*cm.*
Right kidney...	23	5.3	1.95	2.5
Left kidney.....	44	6.0	3.55	3.05

Protocol 19

Right ureteroduodenostomy of three hundred and eighty-one days' standing, showing late renal atrophy. Dog 21-38. Male, spaniel, weight, 20 pounds.

October 28, 1920. Right ureteroduodenostomy.

November 1, 1921. Urine has been slightly bloody for one week.

November 14, 1921. Dog died early this morning.

Autopsy. Rigor is not present. Animal is still warm. Blood in heart not yet clotted. All tissues are bronze in color. Heart and lungs clear. Abdominal content shows no adhesions. There is no loss of peritoneal sheen. The gall bladder is distended but readily empties into the duodenum. The right ureter runs to the duodenum without kinks or obstructions. There are no adhesions about the site of the ureteroduodenostomy. Fluid injected into the renal pelvis flows into the duodenum with great ease. The right kidney pelvis holds about 3 cc. It is almost completely atrophied and shows very great loss of substance but has a smooth sheen to its surface and presents no evidence of scar formation. The left kidney pelvis holds about 3 cc. also. The left ureter is normal and entirely unobstructed. The urine is cloudy and contains many pus cells. The bladder shows a considerable amount of cystitis. The left kidney has many scars in its cortex and is about normal in size. No further abnormalities are noted.

Kidney measurements

	WEIGHT	LENGTH	WIDTH	DEPTH
	grams	*cm.*	*cm.*	*cm.*
Right kidney....	36.8	6.3	2.7	3.65
Left kidney.....	9.8	4.25	1.4	1.5

Protocol 20

Right ureteroduodenostomy of four hundred and nine days' standing, showing late renal atrophy. Dog 21-37. Male, short-haired black mongrel, weight, 24 pounds.

October 25, 1920. Right ureteroduodenostomy.

October 29, 1921. Blood urea nitrogen, 26 mgm. per 100 cc.; non-protein nitrogen, 50 mgm. per 100 cc.

January 4, 1922. Blood urea nitrogen, 21 mgm. per 100 cc.; non-protein nitrogen, 39 mgm. per 100 cc.

January 7, 1922. Killed with ether.

Autopsy. The abdominal sheen is normal in appearance. There are no adhesions present. The left kidney is large but normal in all other respects. The right kidney is exceedingly small and almost buried in its fatty capsule. Its ureter is little if any larger than normal and issues from a small undilated pelvis, which holds about 1.5 cc. of fluid. The duodenal end projects into the duodenum in a mucosa-covered papilla. Fluid can be forced through this papilla with great ease into the duodenum.

Kidney measurements

	WEIGHT	LENGTH	WIDTH	DEPTH
	grams	*cm.*	*cm.*	*cm.*
Right kidney....	5.4	2.7	1.28	1.4
Left kidney.....	39.0	5.6	2.9	2.9

b. The late changes in the two kidneys following ureteroduodenostomy. If an animal with a successful right ureteroduodenal transplant is allowed to live for one or more years, conditions at autopsy are quite different than when it is sacrificed after two to three months. The right kidney will now be found to have undergone marked or even complete atrophy, whereas the left kidney will be in no way dissimilar to that in the previous experiment. In these atrophic right kidneys the evidence of infection and of hydronephrosis is wanting or very slight, although it cannot be said that one or both have not been factors in hastening the late atrophy. That either or both are not the sole factors seems evident. The ureteral opening into the duodenum is patent and normal and there are no kinks or ureteral obstructions discoverable. In figures 19 and 24 are reproduced photographs of the right atrophic and left hypertrophic kidneys four hundred forty-three and four hundred nine days after right ureteroduodenostomy (protocols 18, 19 and 20), and in figures 21, 22 and 26 are shown microphotographs of these atrophic kidneys.

c. Discussion. The explanation of the early hypertrophy of the right kidney following transplantation of the ureter into the duodenum seems obvious. It is burdened with the same double load as its undisturbed mate and it is natural that it should undergo a similar hypertrophy. But the reason for the late atrophy is not so simple. Progressive degeneration from infection or back pressure as the cause is not entirely eliminated by the absence of evidence of either. In a number of the experiments of short-period transplant an imperfect transplant caused hydronephrosis, sometimes of considerable degree, and it is reasonable to suspect this early back pressure as being the cause

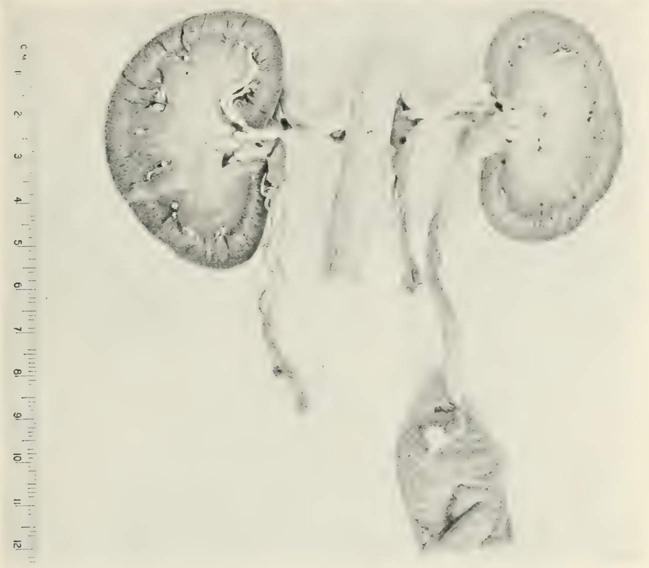


Fig. 18. (Dog 21-35, protocol 17.) Photograph of a beginning late atrophy of the previously hypertrophic right kidney four hundred and forty-three days after right uretero-duodenostomy. The right ureter is not enlarged or dilated and therefore probably never has been. Furthermore ureteral obstruction would be likely to persist if at any time present. The ureteral opening into the duodenum is freely open and is clearly seen in the photograph. The right kidney is definitely smaller than the left hypertrophic one. There is no pelvic dilatation. The cortex and medulla are easily differentiated.

of the late atrophy even when evidence of back pressure at this time is wanting. The marked difference in the histologic appearance of the atrophy of disuse in these kidneys and the degeneration from infection and hydronephrotic atrophy in the others allays to a certain extent such a reasonable suspicion and constitutes the main support of the correctness of our original assumption that the renal tissue does less and less work and so finally atrophies from disuse. But differences in tissue resistance to the injurious action of even a moderate bacterial toxin over a long period as a necessary sequence to differences in tissue activity and inactivity must not be overlooked and would undoubtedly tend to hasten an atrophy of disuse, if nothing more. The appearance of renal fibrosis in some of these late atrophies and the marked interstitial changes present closely simulate the chronic fibrosis

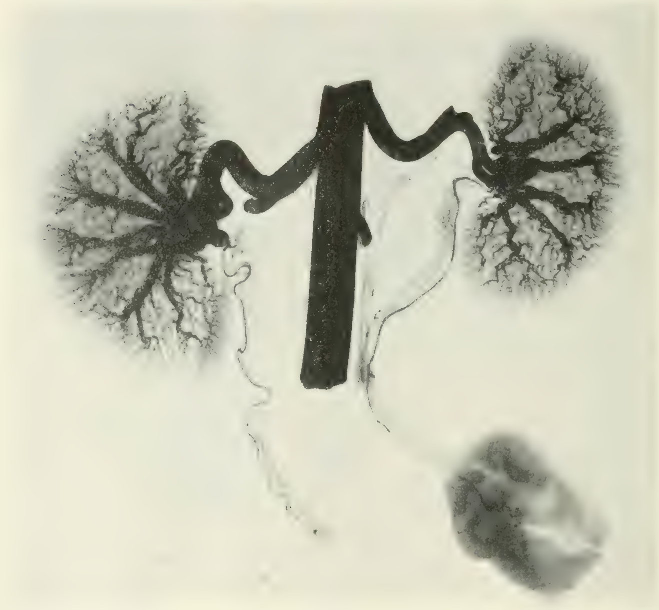


FIG. 19. Photograph of x-ray plate of specimens (Dog 21-35, protocol 17) cut sagittally after arterial injection with barium sulphate. The attenuation of the vascular tree on the right is demonstrated and the ureter does not appear dilated.

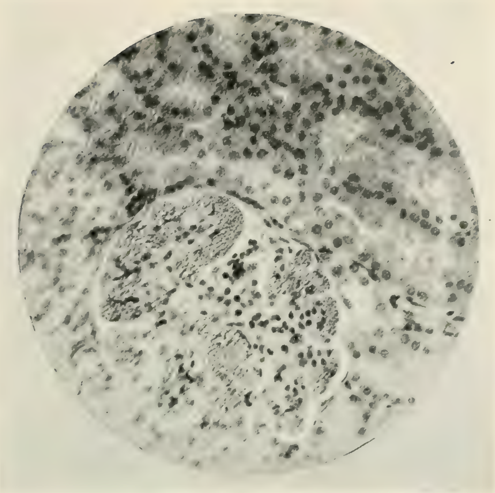


Fig. 20. (Dog 21-35, protocol 17.) Microphotograph (high power) of the hypertrophic left kidney, to be compared to figure 21.

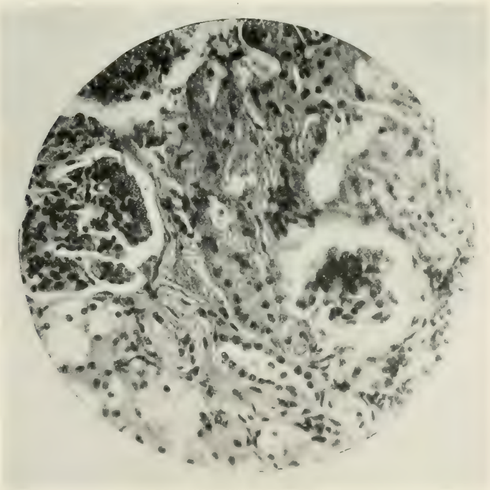


Fig. 21. (Dog. 21-35, protocol 17). Microphotograph (high power) of the late atrophic right kidney, to be compared to figure 20. Shrinkage in the size of cells, interstitial thickening and gnarling of glomeruli are seen.

of old nephritis and arteriosclerotic changes. We therefore hesitate to offer an explanation of the late atrophy after duodenal transplantation. Conditions are not analogous to those in repair hydronephrosis. Competition as a factor is absent. Yet the transplant kidney impresses one as having atrophied more from disuse or diminishing activity than from either back pressure or infection. The almost total destruction that is found in one year would require marked evidence of hydronephrotic atrophy in the one case and of pronounced infection, such as pyonephrosis, in the other. The kidney of dog 21–35, protocol 18 and figure 18 is extremely interesting in this connection as it shows the least amount of atrophy, although this is very definite both in the gross and on microscopic study. The implication of disuse or gradual loss of function as a factor—although it may not be

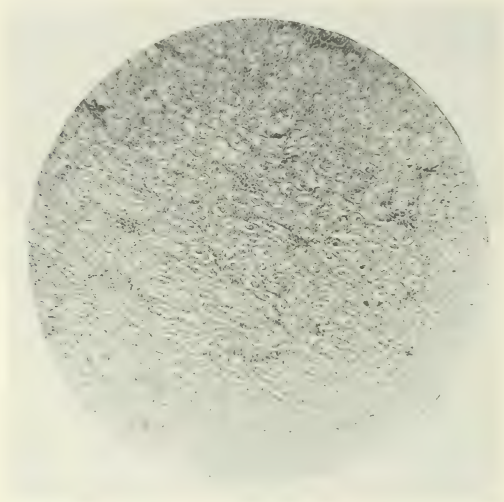


Fig. 22. (Dog 21-38, protocol 18.) Microphotograph of section of right atrophic kidney illustrating marked hyaline degeneration of the tubules.

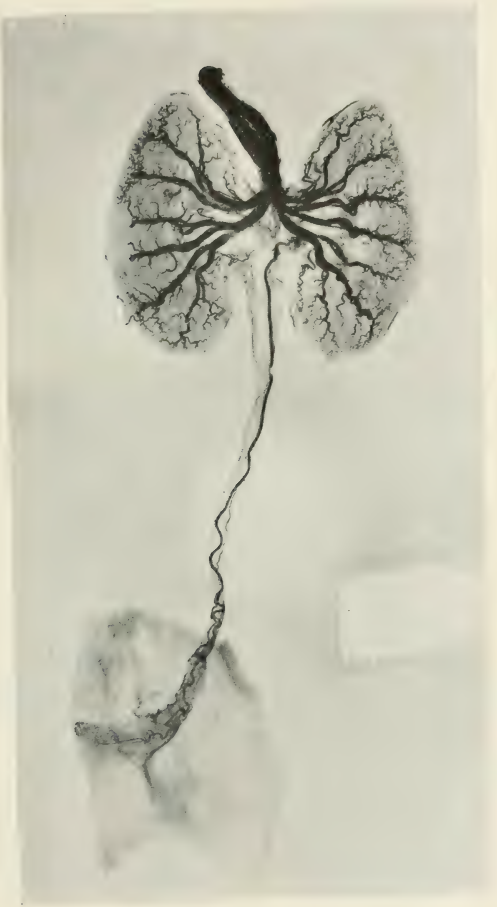


FIG. 23. Photograph of x-ray plate of right kidney (Dog 21-38, protocol 18) cut sagittally after arterial injection with barium sulphate. The shrinkage of blood vessels is marked.

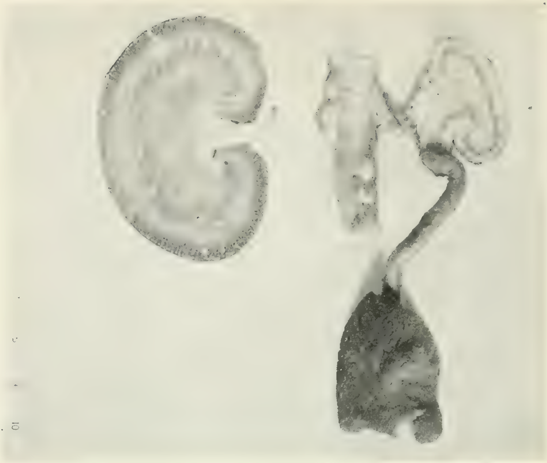


FIG. 24. (Dog 21-37, protocol 19.) Photograph ($\frac{3}{4}$ size) of left and right kidneys 409 days after right ureteroduodenostomy. The left is healthy and hypertrophic. The right side shows evidence of some ureteral dilatation, although the ureteral opening through the duodenal wall is freely patent, but the right kidney is shrunken and markedly atrophic, and this atrophy is totally unlike a degeneration from infection (pyonephrosis) or hydronephrotic atrophy. There is shrinkage in size but with remarkable preservation of kidney shape. Both kidneys have been injected (arterial) with $BaSO_4$ and the left shows rich vascularization whereas the right blood supply is markedly attenuated.

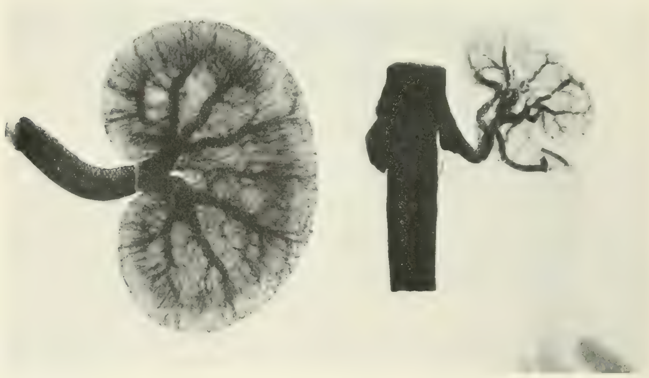


FIG. 25. Photograph of x-ray plate of right (small) and left (large) kidneys (Dog 21-37, protocol 19) cut sagittally after arterial injection with $BaSO_4$. The shrinkage of the arterial system corresponds to that of the parenchyma, both of which are pronounced.

the sole factor—in the late atrophy seems necessary. But how explain the loss of function? How does this kidney learn to work at a slower pace than its divorced fellow? The demand for work would seem to be exactly the same for it as for the undisturbed kidney, inasmuch as they get an equal load in the blood for excretion. Possibly ureteral transplantation is impossible without

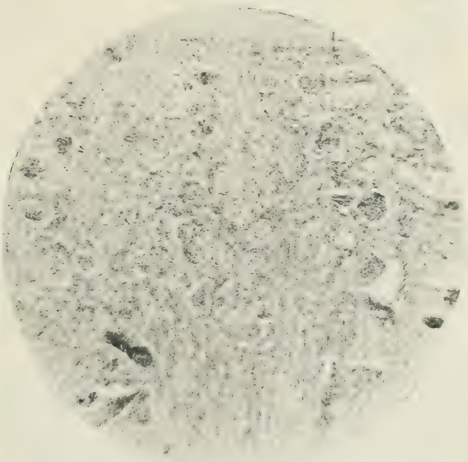
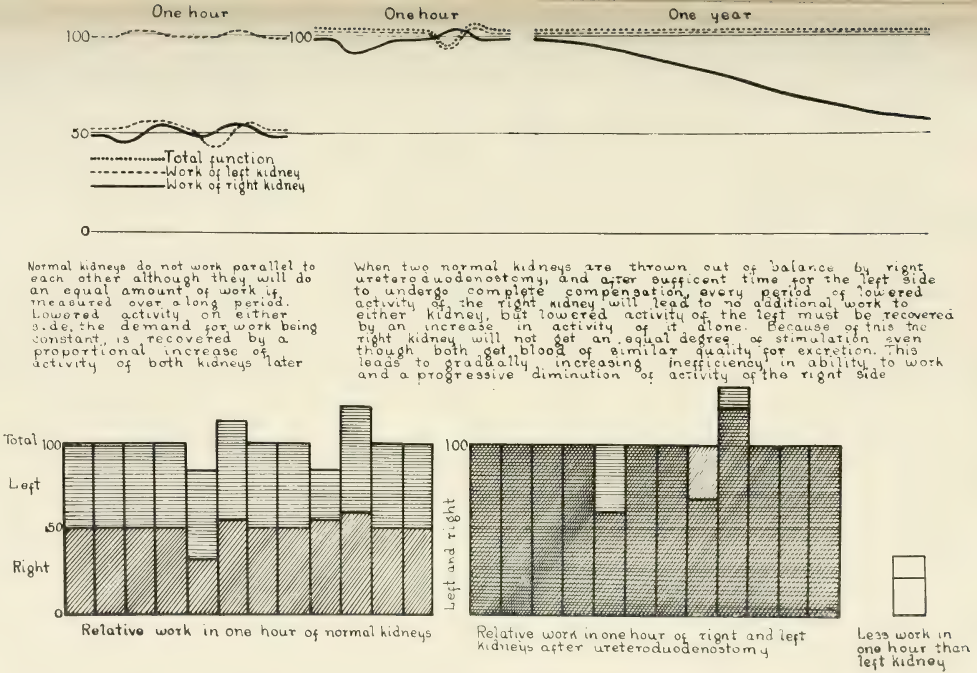


FIG. 26. (Dog 21-37, right kidney.) Microphotograph (low power). Histologic evidence of disuse atrophy, the late atrophy that occurs after ureteroduodenostomy. Marked fibrosis and particularly well marked hyaline degeneration of glomeruli. Tubular system replaced by fibrous strands. Gives the appearance of a chronic fibrosis of Bright's disease.

some renal damage, either by back pressure or infection, and this initial injury puts the transplant kidney qualitatively to a disadvantage. If this were true you would have difficulty in explaining the early compensatory hypertrophy of this transplanted kidney. Compensatory hypertrophy is marked even in those kidneys with definite hydronephrosis from an imperfect duodenal transplantation. We can think of the following explanation as a probable factor.

Two normal kidneys may be likened to a well paired team of horses. The stay chains of the double-tree may be tightened periodically, first on one side and then on the other, as one or the other opposite member of the team slacks up in pulling the load. But in the course of the day each horse will have done an equal share of the work. So with two kidneys. Work is not continuously and uniformly equal. This would seem to be a well established fact, as first pointed out with emphasis by Albarroan. First one and then the other slacks up for a short period. But the work undone must be finished and the added load is put up to both kidneys equally, so that when measured over a long period they will each have done a proportionate share of the total work. In the experiment with right ureteroduodenal transplantation the two kidneys are put out of balance, and now they cannot be compared to a team of horses. Coöperative competition is removed. The stay chain on the left is continuously strained and whenever the left kidney slacks in its efforts it alone must later make up for its short vacation. It gets no aid from its divorced fellow. Renal work is completely dependent upon it, and it alone. But the stay chain on the right may be slackened with impunity. No additional burden will result to either the right or the left kidney thereby, and the right side will gradually take a longer and longer rest, or will do work less and less efficiently, until it will finally atrophy from disuse. In the earlier periods, however, before left hypertrophy has occurred, the double stimulus in the blood activates the right kidney to a similar degree. It has an ever present demand for more work than it is doing and its functional activity is stimulated to the optimum, so that in consequence it hypertrophies. With the completion of a perfect compensatory hypertrophy on the left side the added stimulus is now removed from the blood and the right kidney begins to suffer from the slow injurious effects of inaction in consequence of normal rest periods, which when prolonged lead to its final and complete atrophy. This idea for the sake of clearness may be diagrammatically represented as in figure 27.



Normal kidneys do not work parallel to each other although they will do an equal amount of work if measured over a long period. Lowered activity on either side, the demand for work being constant, is recovered by a proportional increase of activity of both kidneys later

When two normal kidneys are thrown out of balance by right ureteroduodenostomy, and after sufficent time for the left side to undergo complete compensation, every period of lowered activity of the right kidney will lead to no additional work to either kidney, but lowered activity of the left must be recovered by an increase in activity of it alone. Because of this the right kidney will not get an equal degree of stimulation even though both get blood of similar quality for excretion. This leads to gradually increasing inefficiency in ability to work and a progressive diminution of activity of the right side

Fig. 27. Diagram to illustrate possible explanation of late atrophy after uretero-duodenostomy as largely a disuse atrophy. Competition is indirect though not entirely removed. Injury to the transplanted kidney would create an unequal efficiency. But provided an injury occurred would an unequal variation in function lead to a disuse atrophy?

C. Summary of experimental study

The above experiments demonstrate the occurrence of renal counterbalance, illustrate the difference between a functional and an anatomical compensation in renal work, and show the slow progressive nature of the latter, which may be complete only after many months or years. They emphasize the fact that the anatomical is the only type of counterbalance for serious consideration surgically. In the establishment of this anatomical counterbalance four factors are found to be active, of which two are primary, renal reserve power and renal competition; and two are secondary, compensatory hypertrophy and disuse atrophy.

Renal reserve refers to the potential strength or power of renal tissue to perform more work on demand. Its native or static reserve is limited and when the demand surpasses this limit, hypertrophy to a necessary though limited degree occurs. This ability to hypertrophy on demand may be called the compensatory reserve of renal tissue. But if this compensatory ability is competent the physiological inertia of renal cells will overcompensate and thus acquire a comfortable margin of strength for an occasional overload, which may be termed the acquired reserve.

The usual anatomical division of renal tissue into symmetrical and equal halves and the frequency of unilateral injury signalize the surgical importance of renal reserve. Excretory work and renal supply ordinarily balance. The former considered as a whole is fairly constant but alterations in the supply, by disease, injury or operation, are frequent and the anatomical separation of the renal mass accentuates these changes. The anatomical counterbalance may be relative. Complete destruction on one side has not occurred. Changes in the supply on one side, therefore, will either diminish or increase the demand on the other as well as on itself, since they will both have blood similarly loaded with excretory substances. In the process of establishing a new counterbalance of renal tissue on the right and left side as a result of this alteration there will be an active competition between their relative reserve strengths, which may lead to a progressive hypertrophy on one side and a regressive atrophy on the other.

When all renal portions on the right and left kidneys have a relatively similar reserve power they will undergo proportional changes in the process of anatomical counterbalance secondary to alterations in the supply or demand. There will be an even reapportionment of functional activity and a coöperative type of anatomical compensation.

When the renal substance that is thrown out of balance has dissimilar reserve power and potential strength, competition renders counterbalance also disproportional, and that side or portion with the bigger and healthier reserve will show the greater compensation, the weaker side gaining little or nothing in functional strength by reason of the increased demand. The stronger side will gradually outstrip the weaker and by compensatory hypertrophy may be so capable of counterbalance as to render the work of its weak assistant unnecessary, disuse atrophy of which will then follow.

Renal counterbalance according to this conception may be represented diagrammatically and in illustration of the four types of animal experiments, as in figure 28, showing the changes in supply and demand and the renal substitutions in consequence.

IV. CLINICAL STUDY

A. *Introduction*

Anyone at all familiar with renal surgery will recall many opportunities to observe and study renal reserve and compensatory hypertrophy. Experience with polycystic kidneys teaches with how small an amount of renal tissue an individual may live in apparent health. But these kidneys are working to the height of their ability, are unable to hypertrophy and break down when a demand for more work arises. Similar conditions occur in cases of chronic nephritis. It is of interest in this connection to mention the beneficial effect of morcellement in the one case and of decapsulation in the other. Relief of intrarenal pressure in both allows an increased blood supply and a compensatory group hypertrophy to occur, and in consequence an

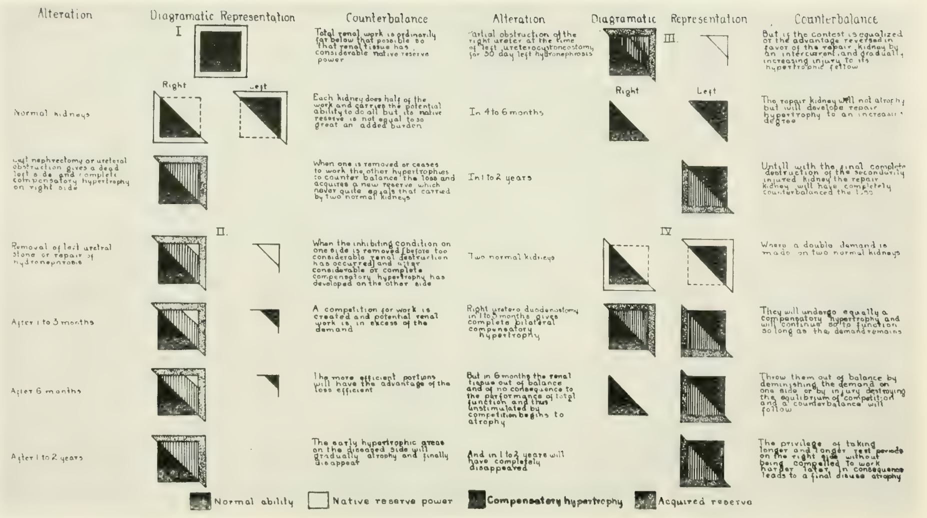

Alteration
Diagramatic Representation
Counterbalance
I
Total renal work is ordinarily far below that possible so that renal tissue has considerable native reserve power
Right
Left
Normal kidneys
Each kidney does half of the work and carries the potential ability to do all but its native reserve is not equal to so great an added burden
Left nephrectomy or ureteral obstruction gives a dead left side and complete compensatory hypertrophy on right side
When one is removed or ceases to work the other hypertrophies to counter balance the loss and acquires a new reserve which never quite equals that carried by two normal kidneys
II.
Removal of left uretral stone or repair of hydronephrosis
When the inhibiting condition on one side is removed [before too considerable renal destruction has occurred] and after considerable or complete compensatory hypertrophy has developed on the other side
After 1 to 3 months
A competition for work is created and potential renal work is in excess of the demand
After 6 months
The more efficient portions will have the advantage of the less efficient
After 1 to 2 years
The early hypertrophic areas on the diseased side will gradually atrophy and finally disappear
Alteration
Diagramatic Representation
Counterbalance
Partial obstruction of the right ureter at the time of left ureterocystoneostomy for 30 day left hydronephrosis
III.
But if the contest is equalized or the advantage reversed in favor of the repair kidney by an intercurrent, and gradually increasing injury to its hypertrophic fellow
Right
Left
In 4 to 6 months
The repair kidney will not atrophy but will develope repair hypertrophy to an increasing degree
In 1 to 2 years
Untill with the final complete destruction of the secondarily injured kidney the repair kidney will have completely counterbalanced the loss
IV
Two normal kidneys
Where a double demand is made on two normal kidneys
Right uretero duodenostomy in 1 to 3 months gives complete bilateral compensatory hypertrophy
They will undergo equally a compensatory hypertrophy and will continue so to function so long as the demand remains
But in 6 months the renal tissue out of balance and of no consequence to the performance of total function and thus unstimulated by competition begins to atrophy
Throw them out of balance by deminishing the demand on one side or by injury destroying the equlibrium of competition and a counterbalance will follow
And in 1 to 2 years will have completely disappeared
The privilege of taking longer and longer rest periods on the right side without being compelled to work harder later, in consequence leads to a final disuse atrophy
Normal ability
Native reserve power
Compensatory hypertrophy
Acquired reserve

FIG. 28. Diagram to illustrate the several experimental alterations of renal supply and the resultant counterbalance of the remaining renal substance.

improvement in function. Pousson (19) has mentioned this as the more probable explanation of the action of Edebohls' operation than that the kidney circulation is improved by a new capsular blood supply.

The clinical problem in relation to surgery is, first, the determination of the renal reserve and ability to hypertrophy of the respective renal masses, and, where the surgical preservation of tissue is in point, the further determination of the probable action of competition and renal atrophy in the final counterbalance. For relative functional study those tests that put renal tissue under strain would seem to be more valuable in this connection. We lack such a test with ureteral catheterization and have to rely upon a consideration of the pathological processes as best we can determine them, together with the relative functions shown. It will be seen that renal counterbalance, with its factors of reserve and competition, is a much broader basis for the consideration of problems of renal surgery than relative functions alone, since it presupposes an attempted foreknowledge of the final result.

V. REPORT OF CORES

When an attempt is made to apply these principles clinically the confusion between a functional and an anatomical counterbalance is irremediable in view of inability to correlate functional findings with anatomical conditions. We have no renal tests that denote renal reserve and cannot distinguish the degree or even existence of native, compensatory or acquire reserve power. But from operative findings, material and results many clinical examples of the process of compensation as well as of competition may be collected.

Case 1. P. C. 609. Male, age twenty-two. Referred by Dr. H. W. Allen.

Complaint. Renal colic, passage of gravel.

Family history. Mother living and well; age fifty. While she was pregnant with patient she had attacks of colic, after which she passed blood in urine. Father has high blood pressure. Brothers (two), one sent to Arizona for possible tuberculosis; one sick with fever for a long time. Living sister had a facial paralysis, age twenty-one; cleared in a few weeks. No miscarriages in family. Grandmother died of tuberculosis; spent winters with patient's family when he was young. Grandfather died of heart trouble. Aunt had a tumor of uterus.

Past history. Negative.

Diseases: Chicken-pox, mumps, measles. Tonsilitis and sore throats almost continuously five to six years ago. Erysipelas 1913, from which present illness began. Otitis media.

Accidents: Left arm broken. Sprained right wrist.

Operations: Tonsillectomy. Still gets sore throats, less severe.

Habits: Negative.

Past illness. Sore throat 1913, three days later erysipelas in face, which became chronic. Second severe attack two months later, at which time had optic nerve inflammation. Streptococcus vaccine. Albumin in urine; no crystals. In 1915, had first attack of colic; awakened with severe cramp over scrotum. Three months later second attack, gradually increasing to climax, with vomiting, sweating, cramp in toes. No chill; lasted three to ten hours. Left quickly; soreness remained in back. Other attacks November, February, May, August, free for nine months, to July, 1917. Doctor found swelling and tenderness in upper lumbar region on left. At present patient has no complaints. Is feeling perfectly healthy, has had no pains except occasionally all his joints hurt and are tender to touch. No swelling, fever or redness. His hands and feet perspire a great deal.

Central nervous system. Severe headaches back of eye, nausea relieved by vomiting.

Cardiac respiratory. Sore throats. Dr. Moffitt found heart murmur. Blood pressure 140.

Gastroin testinal. Negative.

Genito-urinary. Noted. For nine years, has had periods of frequency and sensation as if not finished, occasional burning on urination, which soon passes away. No pain; slight increased frequency daily. Nocturia once in early morning just before arising. Urinary, otherwise negative.

Physical examination. General negative.

Abdomen: Flat tympanitic, soft. No masses; no tenderness.

Kidneys: Both kidneys palpable; the right is felt down to the level of umbilicus. Left not so distinctly felt. Spleen not felt.

Genitalia: Left complete inguinal hernia. Contains intestines. Ring admits two fingers. Also left varicocele. Patient has a slight itching eczema in groins around scrotum and anus. Flat, reddened in color.

Extremities: Negative reflexes:	*R.*	*C.*
Patellar	+	+
Achilles	+	+
Babinski	−	−

December 21, 1917. Phthalein: First, 40; second, 15; total, 55 per cent. Wassermann, negative. Complement fixation of blood for tuberculosis, weak +.

December 25, 1917. Severe pain in right kidney region, radiating down to groin. Tenderness to pressure.

December 29, 1917. *Cystoscopy:* Left ureter dilated. Phthalein intervenous.

First fifteen minutes, right transvesically.

Appearance time, two minutes. 15%. Left, trace.

X-ray: Left uretero-pyelogram taken with thorium nitrate (fig. 29). Dilated pelvis of kidney and ureter. Major calyces large and bulbous. Minor calyces obliterated. Dilated ureter describes an S-figure. Following pyelography patient had severe reaction with partial anuria and jaundice, probably due to infectious cholangitis. The seriousness of the anuria and renal disturbance is graphically shown by the following blood retention and phthalein tests:

January 4, 1918. Urea nitrogen, 168.
January 15, 1918. Urea nitrogen, 165.
January 16, 1918. Urea nitrogen, 200.
January 17, 1918. Urea nitrogen, 214.
January 18, 1918. Urea nitrogen, 144.
January 21, 1918. Urea nitrogen, 140.
January 23, 1918. Urea nitrogen, 112.
January 25, 1918. Urea nitrogen, 119.
February 1, 1918. Urea nitrogen, 16.

January 22, 1918. Phthalein intramuscularly, first, 1; second, 4; total, 5.

January 25, 1918. Phthalein intramuscularly, first, 1; second, 4; total, 5.

January 29, 1918. Phthalein intramuscularly, first, 11; second, 7; total, 18.

February 1, 1918. Phthalein intramuscularly, first, 11; second, 7; total, 18.

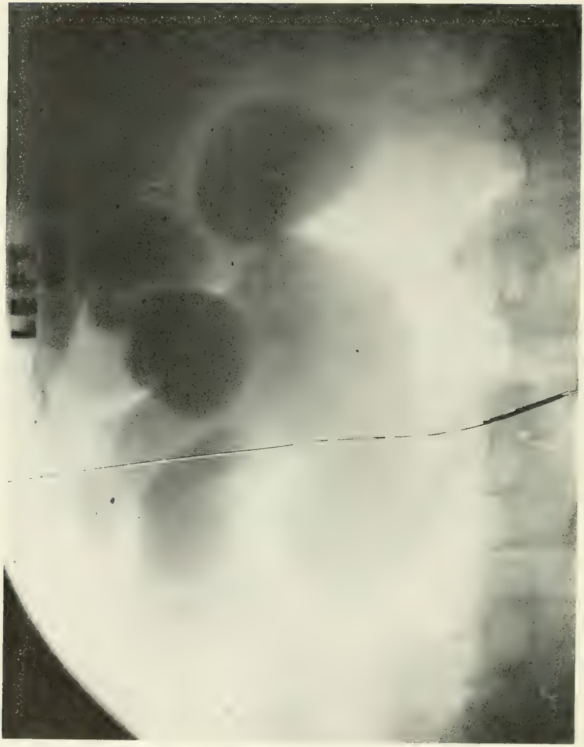


Fig. 29. The degree of hydronephrosis in this pyelogram is so apparent that attempts of conservation would seem absolutely hopeless. This is true provided the opposite kidney is healthy and compensatory. But when there is a distinct demand for even this badly diseased renal tissue to do its utmost, as in cases 6 and 7, figures 37 and 38, conservation may give most surprising and wonderful results.

February 3, 1918. Phthalein intramuscularly, first, 13; second, 8; total, 21.

February 7, 1918. Phthalein intramuscularly, first, 14; second, 14; total, 28.

April 10, 1918. Phthalein intramuscularly, first, 35; second, 25; total, 60.

January 10, 1918. Urine: Staphylococcus albus. Guinea-pig did not die. Urine 1009, albumin. H. T., many pus and epithelial cells. Blood pressure, 120–80. Stool benzidine, +.

January 16, 1918. Eye examination: M. M. 0^2 congested; slight mucoid discharge. Blood vessels full and nerve head and retina slightly hyperemic. Otherwise negative.

September 20, 1918. Cystoscopy: Kidney findings:

Right	*Left*	*Transvesical*
0 R. B. C.	10 qhd.	0
0 W. B. C.	60–75 qhd.	Very many
0 Casts	0	0
Few epithel.	Few	Few
0 Organ.	0	0

Cultures: Taken of bladder and right and left kidney, search for tubercle bacilli and guinea pig inoculation.

Cultures taken of urine (September 20):

Bladder: Growth (B. fecalis alk.).

Right kidney: No growth.

Left kidney: Growth (staphylococcus albus).

No organisms microscopically; no acid fast bacilli. Guinea-pigs were inoculated.

Discharged: Left hydronephrosis. To return for nephrectomy.

October 25, 1919. Readmitted. Cystoscopy postponed. Condition: Same.

October 28, 1919. Readmitted. Cystoscopy: Kidney findings:

Right	*Left*	*Transvesical*
Numerous R. B. C.	Not obtainable	Rare
1 q 3 hd. W. B. C.	Catheter plugged	100 qhd.
0 casts		0
Few epithel.		Rare
0 organisms		0

Cultures: Bladder, right kidney, left kidney.

Phthalein: Right, $3\frac{1}{2}$ appearance time. 25 cc., 20 per cent, first fifteen minutes; 60 cc., 10 per cent second fifteen minutes; 30 per cent total, Left, 10 per cent, 20 cc.

Leakage: 50 cc., 20 per cent phthalein.

Pyelography: Taken of right kidney shows large and normal pelvic outline.

Remarks: Phthalein, October 22: First, 90 cc., 24 per cent; second, 150 cc., 12 per cent; total, 36 per cent. October 25: First, 200 cc., 20 per cent; second, 275 cc., 15 per cent; total, 35 per cent.

October 29, 1919. Discharged.

November 22, 1919. Readmitted. Left ureter dilated with ureteral catheter; 560 cc. urine removed through this catheter, coming spontaneously in fast drops; a continuous stream obtained on pressure over the ureter of this side. Urine from left kidney shows few red blood cells and pus cells; rare epithelial cells; no organisms or casts.

November 24, 1919. Discharged. *Diagnosis:* Left hydronephrosis. Hydroureter with stricture.

December 18, 1919. Readmitted. Cystoscopy: Kidney findings:

Right not catheterized	*Left*	*Transvesical*
Red blood cells..................	Did not run	0
White blood cells................		6 qhd.
Casts............................		0
Epithelial cells.................		2 qhd.
Organisms........................		0

Phthalein: Voided specimen at end of one-half hour; transvesical, 50 per cent.

Remarks: Impossible to pass any filaform or catheter beyond strictured area about 6 cm. above left ureteral meatus. No. 7 catheter passed to this point and left in for collection of specimen.

December 19, 1919. Discharged. Not relieved.

January 6, 1920. Readmitted. Cystoscopy: Kidney findings:

Right	*Left*	*Transversial*
Red blood cells................	Abundant	Abundant
White blood cells..............	1–2 per h.d.	Clumped 1–2 per h.d.
Casts..........................	0	0
Epithelial cells...............	0	0
Organisms......................	0	0

	Right
Phthalein:	
Appearance time	3
First fifteen minutes	25
Second fifteen minutes	10
Total	38

Leakage: At the end of thirty minutes, 12.5 per cent.

January 7, 1920. Discharged. Condition: Same.

January 26, 1920. Readmitted. Urine: Specific gravity 18; albumin F. P. T.; sugar 0; white blood cells and red blood cells, 1 per h.d., epithelial cells many, organisms 0. Phthalein intramuscular: First, 50 per cent; second 7.5 per cent; total, 57.5 per cent.

January 29, 1920. *Operation.* Left nephrectomy for hydronephrosis

General condition: Fair.

Operation: Gas and oxygen anesthesia. The skin was prepared with alcohol, ether and biniodid. An incision about 12 cm. long was made parallel to and just below the costal margin, extending from the costovertebral angle toward the umbilicus. The lumbar and lateral abdominal muscles were divided down to and exposing the perirenal fatty capsule of the kidney. There were numerous adhesions about the kidney which was somewhat enlarged. The kidney was separated from its fossa with little difficulty. The various adhesions to it were separated by sharp dissection after being tied with no. 1 chromic catgut. The vessels at the hilus of the kidney were next identified as well as the ureter. A string tape was placed under the ureter and with traction on this the ureter was held up out of the wound. The ureter was cut about 3 cm. below the kidney pelvis, its distal end being tied off with no. 2 chromic catgut. Three large Ferguson kidney clamps were applied to the renal vessels, after which the kidney was cut between two of these clamps. No. 3 chromic ligatures were used to tie off the renal vessels, the Ferguson clamps being successively removed. There was no subsequent hemorrhage. The deep fascia was approximated with running chromic sutures, the subcuticular tissue with no. 1 plain catgut and the skin with black silk and two silk-worm gut retention sutures. Fluff dressings applied.

Condition of patient on leaving operation room: Good.

Specimen consists of kidney preserved in 10 per cent formalin, dimensions 11 x 5.5 x 5 cm., approximately normal in size and shape except for hilus, which is not of normal thickness. The specimen includes about 2 cm. of the first part of the ureter. The capsule is readily stripped from the cortex, except over a few scar-like depressions where it is adherent, and discolored as if slight hemorrhage had occurred here. Radiating from these areas are minute vessels which appear congested and exhibit punctate extravasations along their courses.

The surface of the kidney appears pale, and exhibits a slight degree of lobulation, and in addition the scar-like depressions already noted suggesting underlying pathology.

On section, the cortex appears approximately normal in thickness (0.6 cm.) and its architecture not distorted except for yellowish white areas underlying the superficial scar-like areas and extending all the way through the parenchyma. They resemble infarcts. They are too yellow to be pure fibrous tissue. They are not wedge shaped. The Bowman's capsules are easily seen. In one area only the cortex is slightly infringed upon by a markedly distended calyx. The medulla has been practically obliterated by a chronic hydronephrosis and pyramids markedly distorted. The renal pelvis and major and minor calyces are tremendously distended. The pelvis and ureteral wall are thickened and there is apparently some constriction at the junction of pelvis and ureter. The lining membrane of the ureter is thrown into folds. The membrane of the pelvis presents a greyish white minutely granular appearance, and a few reddish brown spots which are probably either areas of vascular congestion or extravasation.

The kidney was photographed and sections taken from representative areas; one of capsule, parenchyma and wall of distended calyx, and the other of the wall of the pelvis, including proximal portion of ureter with point of constriction.

Microscopic section (consisting of capsule, parenchyma, and wall of distended calyx) shows the following: The capsule is frayed out and somewhat edematous looking. Over the scarred depressions of the cortex the capsule is adherent and the capillaries appear full but not definitely engorged. There is some hemorrhagic extravasation in the capsule, which is probably of mechanical origin.

The yellowish white areas noted in the gross, which compose about a third of the section are seen to consist of dense peritubular infiltration with lymphocytes and connective tissue cells, and changes so marked in the tubules and glomerulus showing partial or complete hyalin transformation. These areas, therefore, consist almost entirely of lymphocytes and connective tissue.

The parenchyma between these yellowish white areas present various abnormal changes. The glomeruli present various changes from normal to complete hyalin transformation. Many of them are so swollen by hyaline changes that the capsule and tuft are in close apposition. In some the space between tuft and capsule is filled with gelatinous or hyalin looking material and there is considerable connective tissue thickening around many of the capsules. The epithelium of the tubules shows cloudy swelling and vacuolization throughout the whole section. Many are filled with hyalin-looking material.

Throughout the section are seen great number of large phagocytic cells and foreign body giant cells, which are grouped about a slightly granular basophilic substance occupying the lumen of the tubules, especially in the scarred areas, even out to the cortex of the kidney. A few of these phagocytic cells are present in the capsule. Whether these cells are transformed cells from the tubular epithelium or wandering cells of connective tissue origin is not clear, but they give the impression of having originated from the tubular epithelium. Cross section of some of the tubules presents, in place of their normal epithelial lining, a lining of these phagocytic cells morphologically similar to the normal epithelial cells, thus strongly suggesting an epithelial origin. A pyelogram had been taken in the hospital and this basophilic material probably represented thorium (?) salts which had been injected in the pelvis for the roentgenographic purposes. The epithelial lining of the pelvis shows a moderate degree of degeneration and is infiltrated by a few lymphocytes and occasional neutrophiles. The submucosa exhibits a moderate degree of lymphocytic infiltration.

The ureter is thickened. The mucosa appears normal. The submucosa shows slight lymphocytic infiltration, also fairly numerous basophilic phagocytic cells like those described above.

Diagnosis: Chronic phylonephritis with foreign body giant cell reaction (probably due to thorium salts). (Dr. G. I. Rusk.)

January 30, 1920. Patient very restless. Aches all over, particularly back and right side. Vomited about 200 cc. clear liquid. Abdominal pain relieved by enema.

February 2, 1920. Wound in excellent condition.

February 6, 1920. Phthalein: First, 60 per cent; second, 10 per cent; total, 70 per cent.

February 8, 1920. Patient feeling well; no pain, no discomfort. Urine contains acid, sugar 0; albumin 0; sediment, few white blood cells and urates.

February 9, 1920. Small discharge of degenerated blood from lower angle of wound today. Examination of wound shows no opening or evidence of infection.

February 10, 1920. Urinalysis, acid, sugar 0; albumin 0; sediment, few white blood cells.

February 11, 1920. *Diagnosis:* Left hydroureter. Left hydronephrosis.

Condition: Markedly improved.

April 14, 1922. This patient has been under observation continuously since discharge from hospital. Repeated functional tests show maintenance of perfectly normal function.

Discussion. Case 1 is an example of the degree of inhibition that disease on one side may exert upon a perfectly capable and healthy partner. The period of serious renal insufficiency following pyelography further emphasizes this interrelationship. The pathologic findings of foreign body giant cells as a probable reaction from thorium used at pyelography is of interest from another standpoint. The quick and complete compensatory hypertrophy following removal of the inhibiting factor by nephrectomy is striking.

Case 2. P. C. 1528. Male, age forty-seven. Referred by Dr. H. C. Moffitt.

Complaint. Abdominal trouble; flat stools; burning and frequency of urination; dizziness when changes position quickly.

Family history. Father died at fifty-nine, rupture of intestines; mother living and well; two sisters living and well; one sister died in infancy. Not married. No family diseases.

Past history. Diseases: Mumps, measles, typhoid malaria, ill six weeks; rheumatism slightly. Appendectomy three years ago for chronic appendicitis.

Venereal denied.

Habits: Coffee 2 daily; tea 2 daily; no alcohol or tobacco.

Sleeps fairly well. Nycturia once often. Bowels constipated; takes physic.

Past illness. As a youngster occasionally had severe stomach-aches and was constipated. Eight years ago developed a severe belly-ache with nausea and vomiting. Had very frequent urination. Could only pass a drop at a time and it smarted. Some fever. Pain seemed to radiate to bladder. Had passed some high colored urine previously, at one time almost blood red but this had been passed a year or two before he had pain. In this first attack he had to be taken to Alameda Hospital where he stayed five days and gradually recovered. Occasionally had slight pain in stomach after this. Often had a backache in lumbar region. Three years ago another severe attack—no trouble with urine this time. Pain developed this time over appendix and then went away for two days, finally returning. Pain very acute, nausea and vomiting, etc. Appendix removed by Dr. Endlow of Chicago. Appendix long and kinked, in region of kidney. Recovered well and gained weight. A few months later had typhoid-malaria. No similar attacks, not so bad, but since often gets slight pains in right lower quadrant. Has some frequent back pain in lumbar region but no radiation to bladder. Bladder has been irritable since, often urine stings and burns and he passes it frequently. No pain radiating to bladder. Occasionally little sticky pain in lower abdomen—pressure in lower abdomen; belches a good deal; no good appetite. Occasionally sour stomach but very little. No vomiting. No mucous in throat. Dizziness bothers him very little; change of position quickly makes him feel queer.

Urine: February 11, 1920: 1020, acid, yellow, cloudy, a trace of albumen, no sugar; sediment, amorphous urates, a large number of pus cells, occasional red blood cell; rare epithelial cell.

Urine: February 11, 1920; Sediment, large number of pus cells; amorphous urates, occasional red blood cell, rare epithelial cell.

Stool: February 11, 1920: Dark brown, solid, formed stool; no parasites or ova; Benzidin negative, starch negative.

Physical examination. Tall, fairly well developed and nourished man. Hair thin, body hair normal. Skin clear.

Eyes: Pupils irregular and small. React sluggishly to light and accommodation. Eyes set in. Eye muscles negative.

Nose: Negative.

Tongue: Pretty clean. Teeth show a lot of dental work, gums in fair shape.

Tonsils: Red and ragged. No general glandular enlargement.

Thyroid: Negative.

Chest: Well formed.

Lungs: Negative.

Heart: Not enlarged, respiratory arrhythmia. Arteries palpable.

Blood pressure: 125/80.

Abdomen: Level and soft. Liver and spleen not felt. Scar of old appendectomy well healed. There is a little bulging below scar as from moderate relaxation of wall. Cecum palpable. No especial tenderness. No hernia, no rigidity.

Genitalia: Left varicocele; otherwise negative.

Extremities: No tremor; no edema or scars. No joint changes. Hands cold and moist. Reflexes equal and active.

Spine: Straight; slightly stiff in lumbar region with a little pain on bending and twisting in this region. Nothing definitely wrong with sacroiliacs.

Impression: Probable adhesions about scar of appendectomy. Slight hypertrophic arthritis of lumbar sacral region.

Wassermann: February 17, 1920, negative.

Gastric analysis: February 17, 1920:

	HCl	*Total*	
Fasting	0	18	Fasting content about 30 cc., mucoid opalescent; material; Benzidin. negative Starch negative
I	25	63	
II	14	60	
III	24	84	
IV	30	42	
V	20	55	
VI	30	105	
VII	55	120	

Teeth: February 19, 1920: Superior left first bicuspid—large area of rarefaction probably result of infectious process.

Inflammatory rheumatism age eight.

Examination (Dr. Moffitt): Arteries a little softer than recorded. Has a good deal of cramping in legs at night, more on the left. Nothing suggesting lightning pains. Veins right upper arm more prominent than left.

Three-glass test—Urine: February 21, 1920: All three glasses cloudy throughout; one or two containing floculent ppt. Sediment 1, large number of pus cells; amorphous urates; several red blood cells; sediment 3, large number of pus cells; amorphousurates; several red blood cells; a trace of albumen.

February 18, 1920. *X-ray:* Gastrointestinal: The lung fields are clear. The heart and arch are negative. There is no delay in the esophagus.

Stomach good tone and position; freely movable; no defects outline. Moderate peristalsis. Pylorus and cap are well outlined. At six hours:

There is no gastric residue. The six-hour meal is in the ileum and ascending colon which are movable as a mass.

Loops of the ileum are not separable from the cecum, and are slightly tender. At nine hours: The meal is in the cecum, ascending and transverse colon. At twenty-four hours: The meal is well distributed through the colon which shows no obvious defect.

Conclusion: Adhesions about the terminal ileum and cecum.

February 25, 1920. *X-ray:* Kidneys, ureters, bladder. There is an irregular calcified shadow in the region of the upper pole of the left kidney. The left kidney is enlarged and low. The right kidney is low but not enlarged. There is also a small rounded shadow on the right side at the region of the junction of the ureter with the pelvis of the kidney, which is probably a stone in the right ureter. The fourth lumbar vertebra on the right shows an irregularity of the superior border, which has the appearance of an old fracture. There is also a small rounded exostosis on the superior border of the third and fourth lumbar vertebrae on the left side.

March 2, 1920. *Bladder:* Red blood cells 0; white blood cells 7 every h.d.; epithelial cells 3 every h.d. Casts 0.

Left kidney: Epithelial cells 8 every h.d.; red blood cells 20 every h.d.; white blood cells 7 every h.d. Casts 1 granular.

Right kidney: Not running.

March 4, 1920. Waxed tipped catheter passed well into left ureter. No scratches obtained. Left lower end of ureter dilated with no. 6 catheter. Catheter halfway up right ureter for urine study and transvesical phthalein. No flow so phthalein not done.

March 23, 1920. Phthalein: First hour, 20 per cent; second hour, 20 per cent; total, 40 per cent.

March 24, 1920. Urine: Specific gravity 1025; acid reaction; very slight trace albumen; white blood cells abundant; red blood cells occasional; epithelial cells +; casts 0; organisms 0.

Cultures of bladder and left kidney showed Staphylococcus albus.

Right kidney, not running.

X-ray: Same as at previous entry. Irregular calcified shadow in region of upper pole of left kidney. Small rounded shadow on the right side at junction of ureter with pelvis of kidney.

March 25, 1920. *Note by Dr. Kruse.* Dr. Hinman finds a stone in the right ureter which is shown by the last x-ray just at the tip of the catheter, completely obstructing the ureter. He dilated the ureter once and tried to get the stone out but failed. He thinks that Saturday a dilatation should be made again in an effort to get the stone from below. Failing this, he recommends operation for removal of this stone the following week, as the kidney is apparently nearly completely blocked off on that side. X-rays and pyelogram of the left kidney show the stone in the upper pole. Some pus was drained from this side. Total phthalein 40 per cent. Dr. Hinman thinks the left kidney will have to be operated for removal of this stone.

March 25, 1920. In view of the complete blocking of the right ureter and the probability that the right kidney function was much decreased, it would seem wiser to remove the calculus from the left kidney, first unless the stone from the right ureter can be removed under papavarinum and dilatation (note by Dr. H. C. Moffitt).

March 29, 1920. Readmitted.

April 1, 1920. Patient has been passing "smoky" urine since March 29. Urine is clearer than this morning. No pain.

Cystoscopy: Kidney findings:

	Right	*Left*	*Transvesical*
Red blood cells		0	22–24 qhd.
White blood cells		3–4 qhd.	Abundant
Casts		0	0
Epithelial cells		Many	3–4 qhd.
Organisms		0	0

Cultures: Bladder.

Phthalein:

Appearance time, urine collected, five and one-half minutes.

First fifteen minutes, transvesically, twenty minutes.

Second fifteen minutes, bladder catheterized at twenty-five minutes.

Total, end of thirty-minute period forty-five minutes; trace.

Leakage, only trace of phthalein.

April 2, 1920. Slight pain in left kidney region this morning.

April 4, 1920. Culture: Staphylococcus albus.

April 6, 1920. *Operation:* General condition: Excellent.

Reason for operation: Stone in right ureter, junction of middle and lower third.

Gas and oxygen anesthesia. The field of operation was prepared with alcohol, ether and biniodid. A small elliptical incision was made about 2 cm. mesial to the right superior iliac crest, a little farther lateral than it would be done for appendectomy. The fascia of the external oblique was med. and divided in the direction of its fibers.

The muscular attachments of the internal oblique and transversalis muscles were next cut through close to the iliac crest so as to give exposure down to the peritoneum. This was not opened but was dissected off from the iliac crest and superior iliac fossa so as to give an approach to the right ureter by the extraperitoneal route. By using deep blade retractors the muscles and peritoneum were divided so as to expose the iliac artery and vein as well as the right ureter in the region of the right sacro-iliac synchondrosis. The ureter was identified and a string tape pad passed around it. The stone was about 3 cm. above the brim of the pelvis. It was easily palpable and was milked down toward the bladder for about 2 cm. so as to be more accessible. The ureter over it was opened for about 1 cm., allowing the stone to be extracted. An attempt at dilatation of the ureter above was not successful although a filiform could be passed up to the kidney pelvis, but a later ureteral catheter could not. The ureter was freed below this point, giving passage of the catheter into the bladder without difficulty. The ureter was closed with no. 0 iodized catgut. A small tube drain was passed down to the site of operation for drainage and brought out in the groin through a stab wound. The wound was completely closed, the muscles, internal oblique and transversalis being sutured with chromic uninterrupted; the fascia of the external oblique was sewed as in a hernioplasty, and the skin was closed with uninterrupted silk. No silkworm gut sutures were used.

Condition of patient on leaving operating room: Good.

April 9, 1920. Considerable gas pain but is now comfortable.

April 12, 1920. Urine: Yellow, cloudy, acid, sugar 0, albumen faint trace, sediment red blood cells 4–6 qhd.; white blood cells 60–70 qhd.; epithelial cells +; casts 0; organisms 0.

April 13, 1920. Skin sutures removed. Considerable serosanguineous oozing from middle of wound.

April 14, 1920. Phthalein: First hour, 45 per cent; second hour, 15 per cent; total, 60 per cent.

April 15, 1920. *Cystoscopy:* Kidney findings:

	Right	*Left*	*Transvesical*
Red blood cells	50–65 qhd.	Not taken	6–8 qhd.
White blood cells	10–12 qhd.		20–25 qhd.
Casts	0		0
Epithelial cells	Few		2–3 qhd.
Organisms	Cocci +		0

Cultures: Bladder, right kidney and left kidney—All Staphylococcus albus.

April 15, 1920. *Culture:* Bladder, right kidney, left kidney—Staphylococcus albus.

April 20, 1920. *Cystoscopy:* Kidney findings:

	Right	*Left*	*Transvesical*
Red blood cells			0
White blood cells			Clumps
Casts			0
Epithelial cells			Few
Organisms			0

Remarks: Right side easily catheterized. No urine obtained. No retention in kidney pelvis. Catheter open and when injected, the fluid came back free. X-ray pyelogram right kidney shows markedly distorted pelvis, major calices much elongated; minor calices blunt and dilated; ureter not dilated.

April 22, 1920. Phthalein: First hour, 35 per cent; second hour, 20 per cent; total, 55 per cent.

Urine: Clear, light yellow; sugar 0; albumen F. P. T.; sediment; red blood cells 0; white blood cells 6–8 qhd., epithelial cells few, casts 0; organisms 0.

April 24, 1920. *Discharged.*

August 16, 1920. Readmitted: Radiograms show one large and about three small stones in upper portion of the left kidney.

August 17, 1920. *Cystoscopy:* Kidney findings:

	Right	*Left*	*Transvesical*
Red blood cells.........	0	25–30	2–3
White blood cells.......	0	5–6	40–50
Casts..................	0	0	0
Epithelial cells.........	0	0	0
Organisms.............	None seen	None seen	Few cocci

Cultures: Bladder, right kidney and left kidney.

Phthalein: Appearance time, 0 cc., two and one-half minutes; first fifteen minutes, 0 cc., 15 per cent; second fifteen minutes, 0 cc., 10 per cent; total, 0 cc., 25 per cent.

Leakage slight, 5 cc., less than 2.5 per cent.

X-ray reports: August 13, pelvis incompletely filled. August 14, multiple areas of calcification in region of right kidney which have appeared since first x-ray plates of February 25, 1920.

August 18, 1920. Phthalein: First hour, 20 per cent; second hour, 7.5 per cent; total 27.5 per cent, two-hour specimen.

Mouth, gums, throat in satisfactory condition.

Heart: Negative.

Blood pressure, 118/85.

August 19, 1920. Phthalein: First, 40 per cent; second, 25 per cent; total, 65 per cent.

Culture: Bladder, Staphylococcus albus; left kidney, Staphylococcus albus; right kidney, sterile.

August 21, 1920. *Operation.* General condition: Good.

Reason for operation: Calculi in the upper portion of the pelvis of the left kidney.

Gas and oxygen anesthesia. The field of operation was prepared with alcohol, ether and biniodid. An incision about 15 cm. long, parallel to and just below the last rib, was made and carried down to the muscle tissue, bleeding points being caught and ligatured with no. 00 chromic. The muscles between this region and the kidney were incised and the perirenal fat was exposed. By blunt dissection with the finger the fat was pulled from about the kidney and it was completely cleaned off and its pedicle isolated. A strong no. 24 soft rubber catheter was placed about the kidney; the catheter was tightened about the kidney pedicle to cut off the blood supply for a few minutes. A flat curved needle threaded with silver wire was passed through the middle of the kidney in its upper pole and by means of a sawing movement, the upper pole of the kidney was split in half in its least vascular portion. The incision was carried down into the upper portion of the pelvis, of the kidney. Five calculi varying in size from about 3 mm. to 1 cm. were removed from the upper portion of the pelvis. At frequent intervals traction was loosened on the catheter, allowing the blood to flow through the kidney a little. The halves of the upper pole of the kidney were now approximated and sutured by means of five mattress sutures of no. 2 chromic. The catheter was removed from the pedicle of the kidney. No bleeding from the kidney occurred. The kidney was placed in its original position. The portion of the perirenal fat was spread out over it again. The deepest fascia was approximated with interrupted sutures of no. 2 chronic catgut. The deep muscle layer was approximated with mattressed sutures of no. 2 chronic catgut, the superficial muscles and fascia were closed with a continuous locked stitch of the same material. A gauze strip pack which had been placed at the lower pole of the kidney was left in. The skin was closed with continuous locked stitch of black silk except at the lower pole of the incision where the tip of the drain protruded.

Condition of patient on leaving operating room: Good.

August 25, 1920. Blood: Urea nitrogen, 40 mgm. per 100 cc.; non-protein nitrogen, 66 mgm. per 100 cc.; creatinine, 2.09 mgm. per 100 cc.

Blood pressure: 124/74.

August 26, 1920. Blood pressure: 121/76. Frontal sinusitis still persists. Wound considerably reddened.

August 27, 1920. Blood pressure 119/80. Wound draining considerable sero-purulent discharge.

Culture: Staphylococcus albus.

August 28, 1920. Blood: Urea nitrogen, 29.0 mgm. per 100 cc.; non-protein nitrogen, 59.0 mgm. per 100 cc.

August 30, 1920. *Culture:* Staphylococcus aurens. Wound very painful yesterday but much better today.

September 6, 1920. Patient steadily improving.

September 9, 1920. Phthalein: First hour, 45 per cent; second hour, 20 per cent; total, 65 per cent.

September 10, 1920. Urine: Slightly cloudy; specific gravity 1008; neutral; albumen 0; sugar 0; casts 0; occasional epithelial cells; red blood cells 0; white blood cells 1–2 h.d.; few rods and cocci.

September 11, 1920. *Cystoscopy:* Kidney findings:

	Right	*Left*	*Transvesical*
Red blood cells		0	0
White blood cells		1–2 h.d.	6–10 h.d.
Casts		0	0
Epithelial cells		2–3 h.d.	1–2 h.d.
Organisms		None seen	Occas. cocci

Cultures: Staphylococcus aureus in both specimens.

Phthalein: Appearance time, 2½ minutes; first fifteen minutes, 15 per cent; second fifteen minutes, 4 per cent; total, 19 per cent.

Leakage, none.

Remarks: Right side catheterized within about 6 cm. from orifice, beyond which it does not pass. Pyelogram was taken.

September 15, 1920. Urine: Staphylococcus aureus in both left kidney and bladder.

September 16, 1920. *Discharged.* In good condition.

Diagnosis. Stone in left kidney.

April 14, 1922. Patient has been under observation and getting intermittent treatment for a chronic prostatitis. The urine has remained clear and he has had no lumbar pain. The cystic calcification of the right kidney is progressing but this dead kidney is causing no disturbance. Recent functional studies show normal total function.

Discussion. Case 2 emphasizes the necessity of early diagnosis and early treatment if one wishes to be successful in the preservation of renal tissue. Where there has already been complete renal destruction, surgical alterations that earlier would have been followed by repair are obviously useless. The difficulty lies in the recognition of the extent of the destruction. A very considerable reserve power may be so inhibited as to give all the evidence of complete destruction. A distinct indication is present in this patient, on account of stones being present in the opposite kidney, of strict conservation, and the attempt to bring about repair seems justified.

Case 3. U. C. H. 5289. Male, age forty-two. Admitted November 24, 1919.

Complaint. Pain over right kidney region.

Family history. Negative.

Past history. Age twenty-five, malaria. Mumps without complications. No typhoid. "Inflammation of bowels," 1897, in bed six weeks.

Systems: Central nervous system, negative; gastrointestinal, negative; cardiac respiratory, negative; genito-urinary, nycturia two or three times.

Venereal: Denies Neisser and lues. During past twelve to fifteen years lost 38 pounds in weight.

Present illness. Began age twenty-five with pain in right side under ribs, accompanied by nausea and vomiting. Since then patient has had recurrent attacks at irregular intervals—every week to as long as two to three months—lasting from one hour to twelve hours; relieved only by morphine. Has never been jaundiced; pains have never radiated to shoulder. Pain is not related to meals, exercise or otherwise; is relieved somewhat by lying on stomach with pressure applied. Sometimes pain leaves as suddenly as it came; at other times disappears gradually. At times before the attack, and even during the attack, he has frequency of urination; small amounts with burning and some pain in head of penis. Has frequently noticed that after disappearance of pain, he has passed large amounts of urine with "pus"—so much pus that urine would hardly run. Constipation or diarrhea during attacks. No blood in stools.

Physical examination. Thin, underdeveloped, undernourished, middle-aged man, lying in bed; apparently in pain.

Skin, sclera, head, hair, ears, nose: Negative.

Eyes: Pupils contracted (after g.$\frac{1}{4}$M.S.); equal round, react to light and darkness; external muscles O.K.

Mouth: Blood stains on lips, tongue and in mouth. Teeth bad shape, snags, caries, many out; pyorrhea. Tongue protrudes in midline with slight tremor; tonsils and pharynx negative.

Neck: No adenopathy.

Chest: Thin, symmetrical, front moves equally with diminished movement right base behind; no abnormal masses or pulsations; retracted supra clav. pressure.

Lungs: Dull to percussion right top front and back; diminished percussion note over remainder right side. Kroenig's area half gone left top. Bronchial breathing right top, less marked below clavicle; breath sounds faint and distant below second rib in front and below angle of scapula behind. Excursion diminished on right.

Heart: Negative to percussion; sounds moderate quality; no murmurs; A_2P_2. Rate 60.

Abdomen: Marked rigidity of muscles on right side; unable to palpate due to failure of coöperation of patient.

Back, genitalia, extremities and reflexes: Negative.

X-rays taken November 20 show stone in pelvic portion of right ureter. Plain plates of kidneys, ureter and bladder otherwise negative.

Urine: All three glasses turbid. Many pus cells. Acid. All +++. sugar 0.

Meatotomy performed.

November 21, 1919. Phthalein: First, 45 per cent; second, 20 per cent; total, 65 per cent.

No. 26 F. sound passed to bladder.

Sterile culture of bladder urine shows Staphylococcus albus. Gram stain. Gram + cocci.

November 25, 1919. Urine: Reaction acid; albumen +++; sugar 0; many red blood cells; few pus cells.

Cystoscopy. Residual urine: 20 cc.

Bladder capacity: 250 cc.
Kidney findings:

Right	*Left*	*Transvesical*
40–60 qhd.	R. B. C. 2–3 qhd.	5–10 qhd.
13–15 qhd.	W. B. C. 0	15–20 qhd.
0	Casts 0	0
1–2 qhd.	Epithel. 1 qhd.	Few
Motile rods	Organisms 0	Motile rods

Cultures: Right kidney, left kidney taken (November 28) Staphylococcus albus in both specimens.

Pyelography: 50 cc. pelvic capacity.

Taken right kidney. Pain reproduced. Same as present illness.

Remarks: Cystoscope enters with ease. Vesical neck edematous, otherwise negative. Vessels of trigone are dilated. Right ureteral orifice is edematous the opening being enlarged and twice size of left. The mucosa surrounding the orifice presents numerous white deposits which float away but still remain attached on introducing the irrigating fluid. The bladder walls show these same deposits. The posterior bladder walls show some coarse trabeculation that do not stretch out on dilating the bladder. Both ureters catheterized with ease. Bladder irrigated with boric through catheter until returned clear. Bladder capacity averages 435 cc. before patient complains of distension. Retention catheter inserted, no. 18.

X-ray report. Pyelogram of right kidney; hydronephrosis, hydroureter; stone in lower ureter (at about brim of pelvis) (fig. 30).

November 26, 1919. Since cystoscopy patient has been unable to void and has had to be relieved of from 500 to 400 cc. of bloody urine per catheter every seven hours. Severe pain from vesical distension.

November 27, 1919. Temperature 38.7°C. Still unable to void, requiring catheterization at about seven-hour intervals.

November 28, 1919. Still unable to void and becoming more difficult to catheterize. No. 24 soft formerly used. No. 18 Caude now introduced with difficulty. Two marked planes of resistance to passage of catheter: (*a*) bulbous urethra; (*b*) prostatic urethra.

Temperature, pulse and respiration have dropped to normal this morning.

Urine is acid, cloudy, small amount of old blood; some bacteria and pus cells.

November 30, 1919. Abdomen distended, bladder nearly to umbilicus; marked tenderness over right lower quadrant. Abdomen too spastic to feel organs. O.K. for anesthetic. Teeth very bad.

December 1, 1919. Temperature, pulse and respiration down to normal. Retention catheterized in place for three days. Irrigations b. i. d. No burning or sharp pains.

December 3, 1919. Teeth extracted.

Phthalein: First specimen, 35 per cent; second specimen, 15 per cent; total, 50 per cent.

Blood: Hemoglobin, 64 per cent, white blood cells, 18,400; red blood cells, normal. N, 77 per cent; SM, 14 per cent; F, 2 per cent; E, 4 per cent; B, 1 per cent; My, 1 per cent.

December 5, 1919. Retention catheter has been out for forty-eight hours. Patient voiding without difficulty. Slight hematuria.

December 6, 1919. *Operation.* General condition: Good except for moderate anemia.

Reason for operation: Right ureteral calculus.

Operation: Gas and oxygen anesthesia. The skin was prepared with alcohol, ether and biniodid. An incision about 6 cm. long was made just to the right of the midline and midway between the symphysis and the umbilicus. The incision was carried on through to the bladder which was identified and separated from the adjoining tissue by means of blunt dissection and the gloved finger. A search was then made for the right ureter and a large vessel which very closely resembled the ureter was found. This was followed superiorly and inferiorly until a branch was found coming from it. The vas deferens was then encountered and it in turn was followed in hope of finding the ureter. Just at the brim of the pelvis a very large ureter about 1.5 cm. in diameter was discovered. This was opened by a longitudinal incision about 1 cm. long and silk traction sutures inserted in the flaps. A ureteral catheter was then passed in turn into the bladder. The portion of the ureter superiorly was found to be obstructed about 6 cm. from the incision. Ureteral dilators were then inserted and the ureter dilated. Rongeur forceps were then used to remove a calculus which was about 1 x 0.5 x 0.5 cm. The incision in the ureter was then closed by means of no. 0 catgut sutures. The posterior sheath of the rectus and muscles were drawn together with chromic no. 2 as well as the subcutaneous tissue. Two tension sutures were inserted and the skin closed with buttonhole black silk sutures. A stab wound was made to the right of the incision for the insertion of a rubber tube drain and one gauze pack.

Condition of patient on leaving operating room: Good.

December 9, 1919. Small amount hemorrhagic drainage from rubber tube. Patient voiding through catheter.

December 12, 1919. Wound healing well.

Urine: Small pus cells, 1020; sugar 0; diac. 0; reaction alkaline; albumen slight trace; red blood cells + + +; cast 0; white blood cells 0; amorphous material.

December 16, 1919. *Cystoscopy.* Kidney findings:

Right	*Left*	*Transvesical*
0	R. B. C.	0
Entire field full	W. B. C.	40 qhd.
0	Casts	0
4 qhd.	Epithel.	4 qhd.
Few rods	Organisms	Many rods
Solid pus		Debris

Cultures: Bladder and right kidney.

Remarks: Right ureter catherized with no. 7 and pelvis instilled with 6 cm. 1 per cent A_gNO_3 and allowed to drain.

Urine: Small pus cells 1020; sugar 0; albumen slight trace; reaction alkaline; pus + + + +; cast 0; white blood cells 0; bacteria, occasional rod; epithelial cells +.

December 19, 1919. Phthalein: First hour, 35 per cent; second hour, 20 per cent; total, 55 per cent.

March 30, 1920. Reëntry.

March 30, 1920. *Cystoscopy.* Kidney findings:

	Right	*Left*	*Transvesical*
Red blood cells	2–3 qhd.	7–8 qhd.	2–3 qhd.
White blood cells	8–10 qhd. + Clumps	Rarely	40–50 qhd.
Casts	0	0	0
Epithelial cells	2–3 qhd.	3–5 qhd.	Occas.
Organisms	Rods +	None found	Rods + + +

Remarks: $AGNO_3$ 1 per cent injected into each pelvis.

March 31, 1920. March 31: Discharged, with advice to return in two weeks.

April 19, 1920. Reëntry: Since discharge in December, 1919, patient has had two attacks, during which he had a violent chill and a high fever. Last one two or three weeks ago.

Heart: Negative.

Blood pressure: 106–70.

Phthalein: First hour, 35 per cent; second hour, 10 per cent; total, 45 per cent.

Urine: Cloudy, slightly yellow, acid; sugar 0; albumen 0; sediment 0; red blood cells 0; white blood cells 60–70 qhd.; epithelial cells +; casts, organisms, rods +++.

April 20, 1920. Right nephrectomy.

General condition: Good.

Reason for operation: Pyonephrosis.

Gas and oxygen anesthesia. The field of operation was prepared with alcohol, ether and biniodid. The usual incision for right nephrectomy was made parallel to and just below the margin of the tenth rib about 10 cm. long.

There was very little subcuticular fatty tissue. The muscles were not very heavy. The fascia and muscles were cut through down to and exposing the capsule of the kidney. Retraction with wide blade retractors brought the kidney well into view. By blunt dissection for the most part the kidney was freed of the few adhesions which were about it. It presented the picture of pyonephrosis. The ureter, however, was very small and seemed to come off almost down in the kidney. The pelvis was not markedly dilated. A ribbon tape was placed about the ureter, traction upon which brought the ureter into view. The renal vessels were identified and Ferguson clamps applied, after which the kidney was cut from its vessels at the hilus. The ureter was cut about 3 cm. from the pelvis. The ureter and kidney vessels were tied with no. 2 chromic catgut ligatures and clamps removed after the ties were placed. There was very little bleeding. The vessels were tied with no. 1 plain catgut. A small gauze strip was placed, as well as two rubber tubes for drainage. The wound was closed about these, no. 1 chromic continuous suture for the muscles and fascia, two silkworm gut retention sutures, no. 1 plain catgut for the subcuticular tissue and black silk in the skin.

Condition of patient on leaving operating room: Good.

Pathological report: Microscopic examination of hydronephrotic kidney shows extensive destruction of the cortex and medulla with irregular foci of lymphocytic infiltration and fibrosis. Numerous hyalin glomeruli and marked hyalin thickening of blood vessels occurs. In between the more or less focal degenerations there are areas of comparatively normal kidney tissue. Such areas show cloudy swelling of the epi-

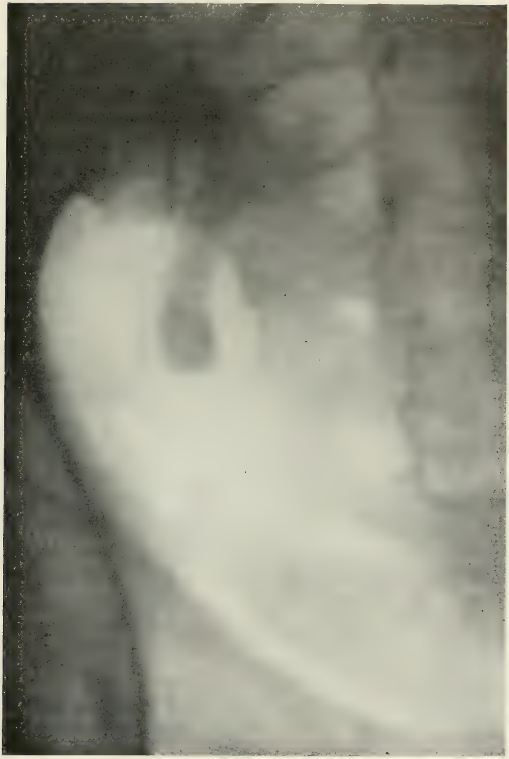


FIG. 30. This ureterogram is similar to that obtained in case 1 and the ureteral stone is about the same in size and position. Removal of the stone in either did not lead to improvement in the function of the corresponding kidney; in case 1, because the stone had so long and completely blocked the ureter as to have destroyed the renal tissue above, and repair was not possible even though there was a demand for it, a stone being present in the opposite kidney; and in case 2, because injury, while not complete, was sufficient to have caused compensatory changes in the opposite kidney and a demand for the repair of this injured side was wanting after removal of the stone.

thelium. The pelvis is lined by the usual epithelium, beneath which in some areas there is fibrosis and edema and sparsely scattered inflammatory cells. In other areas there is marked inflammatory infiltration beneath the epithelium, principally lymphocytic, but scattered in this layer are fairly numerous giant cells of foreign body type and large

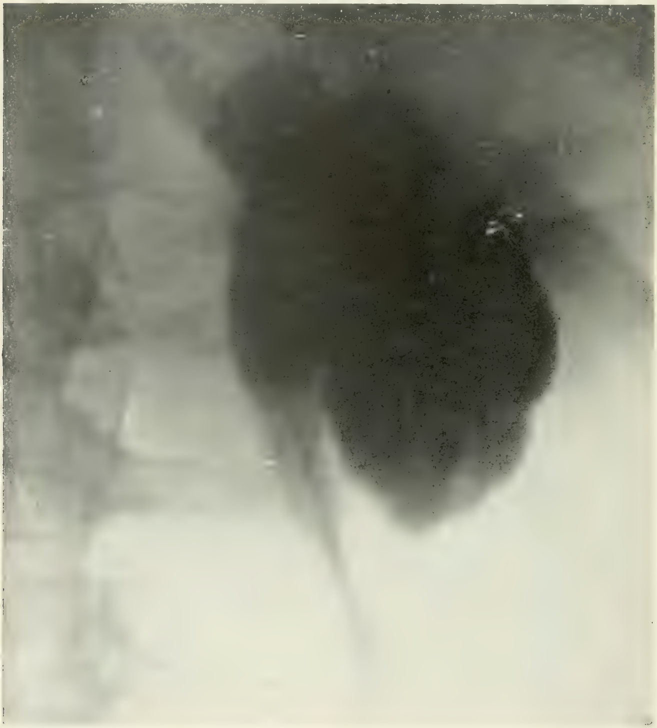


Fig. 31. Pyelogram of the left kidney showing relative small hydronephrosis as compared to figures 37 and 38, and yet this kidney whose obstruction undoubtedly was removed by ureterolithotomy showed less repair than the others subjected to technically difficult plastic operations. The reason is absence of demand for repair of this left kidney.

mononuclear phagocytic cells staining a peculiar purplish gray, the same color that the giant cells stain. This reaction is apparently associated with thorium injection.

Diagnosis. Chronic phylonephritis with hydronephrotic atrophy. Thorium reaction in pelvis.

April 21–30, 1920. General condition: Good.

May 3, 1920. Phthalein: First hour, 20 per cent; second hour, 30 per cent; total, 50 per cent.

May 4, 1920. Discharged.

Condition: Well.

April 1, 1922. Patient in a letter states that he has remained perfectly well.

Discussion. Case 3 is analogous to type 3 in experimental hydronephrosis. Competition between the capable and hypertrophic left kidney and the infected hydronephrotic one, was leading to the gradual and ultimate destruction of the latter. It illustrates the small likelihood of success in repair operations for unilateral conditions when the opposite kidney is compensatory and healthy.

Case 4. P. C. 764. Woman, age thirty-five. Referred by Dr. Max Rothschild and Dr. J. Wilson Shields.

Complaint. Intermittent fever and pyuria.

Family history. Irrelevant.

Past history. Patient has always enjoyed excellent health up to a short time following her last childbirth in May, 1915. There has been no history of focal infection such as abscessed teeth or tonsillitis. Following her childbirth, she had intermittent attacks of chills and fever which were attributed definitely to an infection in the broad ligament of the left side. At this time the urine was normal. A little later in May, examinations of the urine showed a bacilluria due to a peculiar type of colon bacillus with a few polynuclear cells and a few hyaline casts. Blood culture at this time was negative. Spleen was large and soft and there were marked hemorrhoids which were not infected. Her general poor condition continued; the hemorroids troublesome and she complained of pain in the neck. In August, 1917, urine still showed a slight trace of albumin with a number of white cells and bacteria. Patient was treated by rest in bed, regulation of diet, control of the bowels in a attempt to relieve urinary infection. The latter part of

1917, hemorrhoids were removed by operation. Complete gastro-intestinal studies were negative with respect to gall bladder or other intestinal focus.

The phthalein output in March, 1918, was 75 per cent in two hours. At this time infection was much more pronounced on the right side than on the left as shown microscopically, but the phthalein outputs were approximately equal. In June, the phthalein output was 76 per cent and careful bacteriological study of the urine showed a typical anaerogenic paracolon which will grow only upon blood agar plates. Stool specimen examined in June showed normal flora. More complete urologic study in June showed that there was a bilateral hydronephrosis, dilated ureter and movable kidney on the right side, the hydronephrosis on the left being apparently due to a valve-like obstruction at the uretero-pelvic juncture. Infrequent attacks of fever with a dull pain in the right or left side and severe headaches lasting for several days continued to recur.

In September, 1918, uretero-pyelography confirmed the previous finding, showing a rather dilated and tortuous upper right ureter with a hydronephrosis which showed a pelvic capacity of 40 cc. and marked blunting of the minor calyces. Right kidney is quite movable. Left kidney is definitely square-shaped and ureter is not dilated on this side (fig. 32). Left pelvis has a capacity of approximately 45 cc. The phthalein output showed marked variation according to whether the tip of the ureteral catheter had entered the respective pelvis or not, as for example, on March 11, 1919, phthalein with the right catheter not in the right pelvis, but the left one well up in the left pelvis appeared on the left side in five minutes, right side eight minutes; the first fifteen minute output, right side 2 per cent, left side 15 per cent. Whereas, on March 14, with the position of the catheters reversed, the phthalein output on the right was 10 per cent and on the left 5 per cent. Total phthalein on March 13, 1919, was 30 per cent first hour, second hour not taken.

On April 10, total output was 27 per cent first hour, and 11 per cent second hour. Repeated phthalein estimations have shown a very definite and progressive diminution in renal function during the last two or three years.

This résumé of her history lead up to the necessity of consideration of more radical measures, in order to clear up the bilateral infection and the very definite progressive diminution in renal function on both sides. A complete outline of the history with the pyelograms of the right and left side were submitted in consultation to a New York and a Baltimore urologist, as to the advisability of surgical interference and as to whether this should be conservative or radical. The former advised left nephrectomy and we quote this brief abstract from his letter:

. . . . firmly of the opinion that as the parenchyma of the left kidney was probably infected, from the length of time that the infection had existed and the dilatation of the pelvis of the left kidney, that any plastic operation would not be successful, and thought the wisest thing to do would be to remove the left kidney. That he found in a large proportion of cases, operated on by himself and others for plastic operations, almost invariably resulted in a subsequent nephrectomy and he feared a plastic operation in her case would so result."

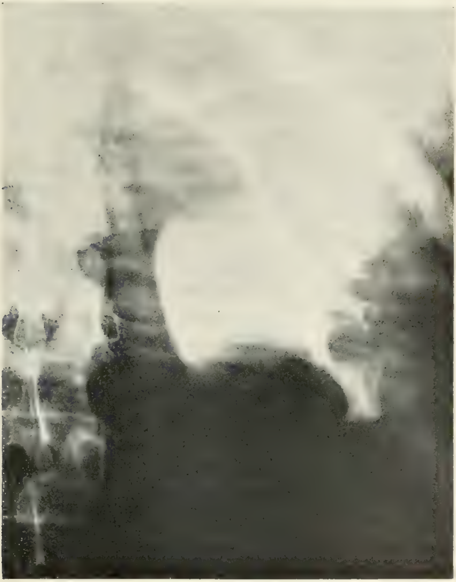


Fig. 32. Pyelogram of the left kidney of case 4 before plastic repair of the uretero-pelvic juncture and nephropexy. A box-shaped pelvis of a capacity of of 45 cc. Infection has been present for years.

The latter urologist advised as follows:

"I have gone over Dr. Hinman's findings in the case of ——— and fully agree with the suggestions for treatment he has outlined. The obstruction which has resulted in the double hydronephrosis is unquestionably at the uretero-pelvic junction and as the hydronephrosis is apparently progressive, it should be corrected. I feel, however, that such preliminary measures as pelvic lavage, etc., should be tried out for the purpose of clearing up the left sided infection if possible. These cases are extremely interesting and in our experience are practically all of inflammatary origin. I feel sure that a plastic at the junction of pelvis and ureter should completely relieve the obstruction."

Continuation of repeated lavage failed to give any permanent relief of the infection and in view of the progressive diminution of the phthalein on both sides, operation was decided by all to be advisable.

April 26, 1919. *Plastic operation on pelvis*—Nephropexy. Gas and oxygen by Dr. Botsford. Iodine preparation; twelfth rib incision; kidney exposed and delivered. Kidney was lobulated and showed considerable hydronephrosis. Pelvis was loose and relaxed and the superior part could be seen to bag considerably below the uretero-pelvic juncture, which was narrowed and constricted. The pelvis was dissected free and the ureters stripped free for a distance of 10 or 12 cm. below. Catgut stay sutures placed in a line directly above the uretero-pelvic juncture and the pelvis opened by a slit in it for about 5 mm. Through this a catheter could be passed into the ureter and there was no evidence of definite stricture, the obstruction apparently being directly due to the valve-like position of the pelvis with reference to its ureter. With stay sutures the ureter and pelvis above were drawn out in a straight direction and an incision made of about 1.5 cm. in length, taking in the upper part of the ureter, the lower part of the related pelvis.

The center of this incision was brought together and the whole sutured with 00 catgut as for pyloroplasty, thus widening the uretero-pelvic juncture considerably. A no. 10 ureteral catheter was cut obliquely with a window about 4 cm. about its end and passed through the pelvic opening down the ureter so that the window lay at the level of the pelvis. The opening about the catheter was then sewed tightly and a piece of fat tissue approximated over it, so as to act as a valve for closure after the catheter was removed. Kidney was then fixed by Brodell stitches with Pagenstetcher on French needles, the upper suture being placed above the twelfth rib; one suture from the mid-part of the kidney to the lumbar muscles and one from the lower pole into the lumbar muscle. A rubber tube drain was placed posteriorly before fixation, and passed alongside the catheter in the ureter and pelvis and exit with it at posterior end of the wound. Muscles closed with continuous no. 2 chromic and double layer; skin closed with continuous silk interlocked.

April 27, 1919. For the last thirty hours the catheter in the ureter has been injected with 5 per cent argyrol, 5 cc. being injected each time every two hours. There has been considerable urinary drainage through the catheter. Urine has been darkly colored with the argyrol. Patient has been unable to void and has had to be catheterized. Catheter was removed without difficulty, drainage tube being left in place.

April 28, 1919. Following removal of catheter there has been practically no urinary drainage. Temperature has remained perfectly normal continuously. Considerable sera from the drainage tube. No hemorrhage.

April 29, 1919. Condition of the wound as on previous note. Less drainage than the day before. Patient still has to be catheterized. Temperature normal.

April 30, 1919. Patient has been extremely nervous and irritable. Condition otherwise satisfactory. Last night at 2 o'clock temperature rose to 99 and this morning at eight was $99\frac{4}{10}$. Wound appears as before. Still less sera than formerly and apparently there has been no urinary drainage since the removal of the ureteral catheter. About 8 cc. of 0.25 per cent Dakin's solution injected into the drainage tube which was then withdrawn for a distance of 5 cm. and cut-off.

May 2, 1919. Very little drainage from tube, which was today completely withdrawn.

May 3, 1919. Stitches removed. Wound looks fairly healthy, some redness at lower end; very little discharge; no urinary leakage.

May 6, 1919. This morning at 2 o'clock temperature again arose and reached 102 during the night. Patient seen late in the afternoon when temperature was still over 100. Urine still cloudy and some soreness in right kidney region. No mass or particular soreness in region of left kidney or in the left hypochondrium. On account of the purulent condition of the urine and temperature, it was thought advisable to immediately catheterize ureters which was done in patient's room by simply moving her to edge of bed. Both ureters easily catheterized. Apparently no retention in left pelvis, but considerable in the right.

Specimens collected and submitted to Dr. K. F. Meyer. Both kidneys lavaged with 2 to 3 cc. of 1 per cent silver nitrate. Specimens, examined by Dr. Meyer, show microscopically: right, few pus cells, many red blood cells and numerous paracolon-like organisms—some in long chains and others in coccoid form. Left urine, microscopically, few pus cells, few red blood cells, abundant organisms, both colon-like and in clusters like staphylococci. Cultures made from both sides.

May 24, 1919. Phthalein: First hour, 27.5 per cent; second hour, 30 per cent; two hours total, 57.5 per cent.

May 26, 1919. Cultural report on urine by Dr. K. Meyer: Pure coli.

June 20, 1919. The temperature rise of May 6 persisted for a period of ten to twelve days, gradually falling by lysis. During this time there was some swelling and tenderness of the right kidney and the urine continued to show organisms and pus, although there at no time were any bladder symptoms. Temperature came to normal and remained so for a period of about ten days and it was thought that patient was on the final road to recovery. On May 28, I left for Atlantic City, and two days later there was a second temperature rise, lasting for three or four days, during which time, the patient showed considerable prostration in the afternoon and also complained of some soreness in the right kidney region. This period of temperature subsided within three or four days to normal temperature and general improvement and a third grade pyrexia occurred lasting several days. Temperature came to normal again on June 14 and after two or three days of normal temperature and general improvement there was a rise again on the 18, to 102.

June 18, 1919. Phthalein: First hour, 30 per cent; second hour, 15 per cent; total, 45 per cent.

Leukocyte count 8600; hemoglobin 65 per cent; red blood cells 3600; 0 blood pressure, on June 20, 110–60.

After consultation with Major Shiels and Dr. Moffitt, who upon careful examination failed to find any evidence of a cause for temperature in the heart, lungs, or any of the viscera, the advisability of ureteral catheterization in order to determine the main source of renal infection, as well as to treat the condition locally was admitted.

June 20, 1919. *Cystoscopic examination.* Bladder negative. Ureteral orifices both somewhat dilated, but similar in appearance as on previous examination. Both ureters easily catheterized, catheters being injected with a steady stream of sterile water for passage into the ureters so as to prevent contamination. No. 6 catheters easily passed to level of renal pelves on each side. There was a continuous steady flow of urine from the left kidney for a short period, about 3 cc. drainage. At first there was no continuous flow from the right but after injection and slight withdrawal of the catheter a steady stream of clear, very pale fluid, which was slightly cloudy and shredded occurred. The left pelvis was injected with 2 cc. 0.5 per cent silver nitrate and the right 5 cc. Catheters were allowed to remain for another ten minutes to allow drainage of this injected solution.

Patient complained of no particular pain or discomfort at the time of the examination. Specimens were taken immediately to Hooper Foundation and examined in conjunction with Dr. K. F. Meyer. After centrifugalization microscopically, right urine showed several red blood cells; few pelvic cells; numerous non-motile rods and clusters of streptococci. Left side, several red blood cells, few pelvic cells, 1 or 2 pus cells and 2 or 3 non-motile rods. Cultures made from each side preparatory to the making of a vaccine.

June 27, 1919. Urines:

Right		*Left*	
Pus	+++	Pus	+
R. B. C.	+	R. B. C.	+++
Casts	0	Casts	0
Org.	++	Org.	
Rods	–	Occas. rod	

Eleven cc. of one per cent Mercuro-chrome. Catheter plugged and left in three minutes.

Eight cc. Mercuro-chrome "220." Catheter plugged and left in two minutes.

June 30, 1919. Phthalein: First hour amount 200 cc., 35 per cent; second hour amount 200 cc., 22 per cent; total, 57 per cent.

Cystoscopic study. Bladder negative on inspection. Both ureteral orifices negative. Right side catheterized with Garceau catheter which was passed high up into the ureter. There was apparently no great retention in the kidney pelvis on this side. Urine came in periodic spurts, slightly cloudy and showed microscopically a number of pus cells and numerous rod-shaped organisms. Submitted to K. Meyer for bacteriologic study. Phthalein injected intravenously appeared through this catheter in four minutes and in fifteen minutes 12.5 per cent was excreted, which shows definite improvement over previous study.

There has been a gradual though slow improvement. The phthaleins have shown definite improvement, all of which has been due to the increase of function in the left kidney. There is apparently a gradual diminishing functioning of the right kidney and the right kidney shows practically all of the urinary infection, only an occasional positive culture being obtained from the urine of the left kidney. On January 19, 1920, catheters were passed into both kidney pelves. There were a few pus cells in the urine from each kidney and many rods in both. Phthalein appeared on both sides in three minutes and the total output in the next fifteen minutes for the right was 15 per cent and for the left 25 per cent. The kidneys were both acting very rapidly and the flow from the left catheter upon withdrawing it into the ureter, was not in any way interrupted or diminished which would seem to demonstrate that the obstruction on the right side is largely at the uretero-pelvic juncture, just as it had been found in the case of the left kidney. On March 3, 1920, the total phthalein output was 50 per cent the first hour and 20 per cent the second hour.

The patient then spent some time in France and during her stay there was under the care of Dr. Heitz-Boyer who treated her infrequently with ureteral catheterization and during the last several months obtained continuously negative cultures from the left kidney, but positive colon bacilli were continuously found in the right.

Pyelograms made by Dr. Heitz-Boyer showed that the obstruction on the left side has been relieved and that apparently the dilatation of the right pelvis is somewhat reduced by repeated catheterization, although this kidney is still quite movable. Following is the cystoscopic report of January 25, and January 26, 1921 made, by Dr. Heitz-Boyer:

Dosage par le methode gazometrique 0.25 0/00

Rein Droit Tube 1,	4.90 0/00
Rein Droit Tube 2,	5.12 0/00
Rein Droit Tube 3,	2.81 0/00
Rein Gauche Tube 1,	16.65 0/00
Rein Gauche Tube 2,	24.08 0/00
Rein Gauche Tube 3,	16.14 0/00
Urine Totale Tube 1,	12.29 0/00
Urine Totale Tube 2,	13.32 0/00

January 26, 1921.

Examen Cyto Bacteriologique.
De L'Urine Vesicale et des reux reins.

Dans les urines remises le lundi 24 Janvier, 1921, nous avons constate:

Urine Totale—Tube 1.

Grandes cellules epitheliales pavimenteuses. Leucocytes pas tres nombreux Pas d'hematies—Sur frottis, tres nombreux bacilles gram negatifs.

Urine Totale—Tube 2.

Grandes cellules epitheliales pavimenteuses. Leucocytes un peu moins nombreux Pas d'hematies. Sur frottis, tres nombreux bacilles gram negatifs.

Rein Droit—Tube 1.

Cellules uretero pyel'ques nombreuses—uelques hematics—Pas de leucocytes—Sur frottis, bacilles gram negatifs pas tres nombreux ayant l'aspect morphologique de Bacillus coli. Uelques elements du petit diplocoque gram positif signales dans les examens precedents.

En route to American, patient was seized with an acute attack of fever in London, where she consulted Dr. Thomson Walker, who, however, did not cystoscope her and make any urologic study. His opinion, however, at that time was as follows:

"In view of the fact that patient is a semi-invalid, unable to take part in ordinary social life, an attempt to get rid of the right pyelitis by operation is justified. Lavage of the renal pelvis has had a prolonged trial and has failed. I recommend right nephrotomy and drainage of the right renal pelvis with continuous irrigation. If the kidney is found unduly movable, it should be fixed. The appendix should be examined through the lumbar wound and if diseased removed."

Patient had no attack after leaving London and was last seen by me on March 15, 1922, when the following note was made.

It would seem definitely proven by the course during the last two years, that the infection in the right kidney will not be eradicated without more radical measures than simple ureteral catheterization and lavage. The appearance of a coccus, as a secondary invader of this kidney renders the necessity of a consideration of a more radical method of treatment pertinent, although regular ureteral catheterization will keep this kidney in a much healthier condition than if neglected. In view of the compensatory hypertrophy of the left kidney and the freedom of infection now for several years, its ability to carry efficiently the total renal work would seem certain, and, with this fact in mind, the quickest cure of the right-sided infection, and probably the safest cure, would be by nephrectomy. This would seem the wisest course also in view of the uncertainty of conservative surgery upon the right kidney at this time, as it is probable that simple kidney fixation would now be insufficient to completely clear up the infection in the right kidney and that nephrotomy with drainage and lavage treatments, in order to clear up this infection (at the time of the nephropexy), carries what would seem to be an unnecessary added risk. The choice of treatment, therefore, lies between a continuation of periodic ureteral catheterization and nephrectomy.

Instrument inserted without particular difficulty and inspection of bladder shows a rather reddened mucosa, the inflammatory reaction being particularly pronounced over the posterior wall. There is no ulceration or oedematous polyp formation, but the bladder wall gives the appearance rather of a profuse granular cystitis. The bladder capacity with cystoscope in place was 380 cc. no. 6 Albarran catheter inserted into each kidney pelvis without meeting obstruction and without difficulty. The relative findings of the right and left kidney were as follows:

Kidney findings	*Right*	*Left*	*Transvesical*
Character of flow......	Continuous	Intermittent	
Macroscopic..........	Slightly cloudy	Clear	
Microscopic..........	Very pale	Highly pigmented	
Red blood cells........	2–4 every h.d.	2030 every h.d.	0
White blood cells......	4050 every h.d.	1–2 every h.d.	6–8 every h.d.
Epithelial cells........	4–6 every h.d.	0	2–4 every h.d.
Casts................	0	0	0
Organisms............	Cocci, rods	0	Cocci, rods
Stained smear.........	Made	Made	

Cultures. Bladder, right kidney show B. coli and left kidney negative.

Phthalein:

Appearance time: Right, 4 minutes; left, 4 minutes.

First fifteen minutes: Right, 12 per cent; left, 27 per cent.

Second fifteen minutes: Right, not done; left, not done.

Leakage: None in bladder specimen at end of fifteen minutes.

Pain: None.

Remarks: T-Stained smear of right urine shows a few rods but very many cocci. Culture of bladder urine obtained by Cather March 11, showed B. coli.

Impression. The relative cystoscopic study indicates that the right kidney is badly infected and the left kidney is free of infection. The right kidney function is definitely reduced or inhibited, whereas, the left kidney is relatively hyperactive. The finding of a large amount of pus from the right kidney, which on stained smears shows the presence of both cocci and bacilli, renders the condition at this time more serious, than when the infection was a pure colon bacillus infection. The seriousness is more with respect as to the proper course of future treatment, than to any immediate necessity of radical treatment.

Case 5. P. C. 961. Woman, age forty-two, married. December 27, 1918. Referred by Dr. E. C. Fleischner.

Complaint. "Chills and fever."

Past history. Patient has borne five children and ever since the last child she has not felt well. For the last eight or nine years, has had considerable sinus trouble both in the ethmoid and frontal sinus. Saturday, without any apparent cause, she began to have burning and frequency of urination and a temperature. Seen by Dr. Fleischner who found on examining the urine that it contained pus and motile bacilli and considered that the patient had a colon bacillus pyelitis. Since then temperature has ranged between 100 and 103 and she has had frequent chills with profuse sweating, with a loss of appetite and infrequent pains low in the back, but no definite lumbar pain or kidney pain. Patient seen December 26, temperature 101, pulse 100, no lumbar or kidney tenderness; right kidney palpable. Urine is cloudy, shows numerous pus cells, hyaline and granular casts, occasional red blood cells, many epithelial cells.

Patient refused to go to the hospital for treatment; a trained nurse put on the case; given bicarbonate of soda for twenty-four hours. After the acute symptoms subsided this was changed to uretropine and acid sodium phosphate.

December 31, 1918. 10.18, cloudy, pus +++, stain pus cells, bacilli.

January 10, 1919. Deep amber, cloudy, 1022. White blood cells +++. Granular casts, rod shaped organisms.

January 20, 1919. Patient began to run a temperature; chilly sensations, pain in the right back. Unable to get nurse for her and entered Adler's Sanitarium today.

Cystoscopic examination. Urethral catheterization was done. Both kidneys were lavaged with 1 per cent silver nitrate.

Urine: Pus +++; no bacilli, casts, red blood cells.

January 22, 1919. Seen by Dr. Jellinek in consultation. Blood count: whites, 5100; reds 4,352,000. Hemoglobin 87 per cent; urine specimen clear. Mic. pus + much less than before.

January 23, 1919. Urine sample: bladder, right and left ureter. Pus cells in each sample examined.

January 25, 1919. Sterile specimen: Few pus cells only. No bacilli seen.

January 27, 1919. Left, no organisms, occasional pus cells, bladder negative. Right, pus cells, blood cells, few rod organisms.

January 29, 1919. Phthalein test: First hour, 22; second hour, 25; total, 47. (Note: The second "hour" test was only for fifty minutes.)

January 31, 1919. Urine sample: Very few pus cells; 0 blood cells, bacilli ++.

February 1, 1919. Phthalein: 10–35 – 7.

February 3, 1919. *Cystoscopic examination.* Both ureters catheterized. Phthalein not done.

Pyelogram: Right kidney shows hydronephrosis with marked kink of the ureter just below the pelvis. The ureter itself is markedly dilated and tortuous, and the dilatation is pronounced below the area of this kink. The plate is not low enough to show lower portion of ureter (fig. 33).

February 3, 1919. *Cystoscopic examination.* Urine from left side negative. Right side few pus cells, occasional rod-shaped organisms. Pyelogram made of the left kidney, shows kidney in normal position with a perfectly normal appearing pelvis, and major and minor calyces. Ureter not dilated, perfectly straight, apparently draining well. Uretrogram made of the right ureter shows stricture in region of iliac crest. The presence of infection, marked ureteral dilatation below the ureteral kink, and a pronounced hydronephrosis above, all indicate the improbability of kidney fixation giving permanent relief. In view of this nephrectomy would seem to be indicated.

February 10, 1919. Phthalein: Left: three minutes; 32 cc., 15 per cent, first fifteen minutes; 30 cc., 8 per cent, second ten minutes.

Right: four minutes; 54 cc., 20 per cent, first fifteen minutes; 34 cc., 7 per cent, second ten minutes.

February 11, 1919. Urine: Microscopically, no organisms.

February 12, 1919. Urine: Bladder: clear, many epithelial cells; no pus; rare bacillus. Right: Negative. Left: Negative.

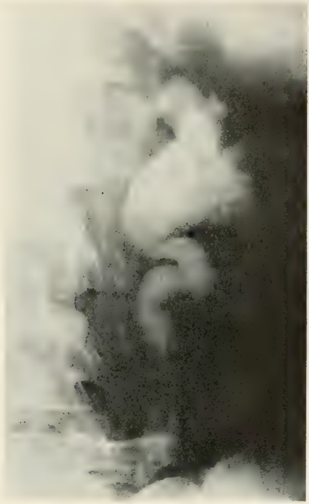


FIG. 33. Pyelogram of right kidney of case 5. Definite obstruction at ureteropelvic juncture. Relieved by plastic surgery and kidney fixation. Chronic invalidism and renal infection with recurrent exacerbations for many years. Complete cure.

February 17, 1919. Phthalein: Right: 17.5 per cent, thirty minutes. Left: appeared three minutes; 27 per cent, thirty minutes. Transvesically no output.

February 28, 1919. *Cystoscopic study.* Bladder negative. Bladder urine shows only occasional pus cell. No blood, rare bacillus. Right ureter catheterized with large 7F without difficulty, meeting no obstruction apparently at the site of previous stricture. Passed easily to the kidney pelvis. Only slight retention. Pelvic capacity as measured with sterile water 45 cc. Phthalein given intravenously appeared through the right catheter in two and one-half minutes. First fifteen minute output 17 per cent. Bladder catheterized at the end of this fifteen-minute period, 22.5 per cent phthalein present.

March 5, 1919. *Nephropexy.* Dilatation right ureter retrograde. Gas, oxygen and ether. Patient placed on kidney pad. Small curved twelfth rib incision, twelfth rib being short and the body form long. Liver was felt low through the incision. Kidney was found low at the lower end of the incision. Kidney was freed of the perirenal fat and by traction upon this delivered into the lumbar wound. There were marked fibrous adhesions all about the kidney which were freed by blunt dissection and scissors. Ureter could be seen at the lower portion and was quite tortuous in its course, the tortuosity being maintained by connective tissue bands surrounding it. These were freed, it being necessary to divide and ligate a small aberrant vessel coursing along the ureter, and which apparently assisted in keeping the ureter kinked.

The kidney was well freed at both its upper and lower pole and left attached only by its pedicle. Ureter was stripped for a distance of 10 cm. from its pelvis, was opened by longitudinal incision and a Bransford-Lewis ureteral dilator passed down the ureter to the level of the bladder. Upon withdrawing and gradually opening this, the point of stricture at the pelvic level could be readily felt and this was gradually dilated. Lewis dilator withdrawn and linear incision in ureter closed with 00 catgut. Continuous suture, a piece of fat being tied over this suture to reinforce it. Puncture drain placed about 2 inches below lower end of lumbar incision through which small rubber tube was passed to area of ureteral opening for drainage in case there should be leakage from this ureteral incision. Twelfth rib freed from subcutaneous tissues. Three Broedell sutures passed for kidney fixation with medium sized Pagenstecher. Kidney fastened up under twelfth rib and to sacrospinalis muscle, being held firmly and satisfactorily in this position. Could not be fastened very high on account of the low position of the muscles closed with two-layer continuous chromic gut no. 2; subcutaneous tissues with interrupted catgut, and skin with locked horsehair.

March 24, 1919. Right: Pus, none; organisms, none. Left: Pus, none; organisms, none.

March 27, 1919. Bacteriological report by Dr. K. Meyer. Kidney urines, negative on both direct and enriched cultures. Bladder shows B. coli.

April 25, 1919. Urine clear, negative microscopically.

March 30, 1919. Patient has been heard from several times since leaving San Francisco for her home in Canada and has remained well with no urinary complaint of any kind until one month ago. At this time she was rather busy and working hard because of illness in her family and as a result complained for a time of pain in the right kidney area which was not at all severe and pain in her bladder. There was associated with it, no frequency, and at this time she had a bad infection in one or both antri. A culture of a catheterized specimen made March 30, 1921, by Dr. G. J. Thomas of Minneapolis was negative and microscopic study of specimen was negative for pus.

Discussion. Cases 4 and 5 illustrate the success of repair operations but emphasize the grave danger of delay in bilateral disease in the performance of the repair procedure on the opposite kidney after successful repair on one side. The surgical problem after the delay although it is justified has led to the serious consideration of radical right nephrectomy in cure of rather than simple nephropexy, as originally indicated. A cure seems unlikely unless one or the other of these measures is adopted.

Case 6. Age twenty-six. Admitted January 15, 1920.

Complaint. Pain in right side.

Family history. Father dead, age forty-nine, typhoid. Mother, living and well; no brothers; three sisters living and well, two died in infancy. Never pregnant; married four years. Wants children. No history of tuberculosis, cancer; no kidney trouble.

Past history. Diseases: Measles, mumps, chicken-pox, whooping cough, bronchitis, tonsillitis. None since fifteen years ago. No rheumatism or "growing pains" in childhood.

Operation: On turbinate.

Accidents: Clavicle broken seven years ago.

Habits: Coffee 0; tea 2; no drugs or alcohol.

Past illness. Menstrual began age thirteen. Regular 28/5. Pain in back and side first days, relieved by flow; occasional headaches with periods.

Patient has always been in good health except as a child she had frequent urination. Eight years ago she fell. This was followed by a pain in right side. Was diagnosed appendicitis. This soon left. In September, 1918, while working hard, noticed it again. Free again. In April, 1919, took a two months' auto trip; pain recurred. Six years ago after fall she felt a lump in her right side. Is always a dull, aching.

more or less constant pain, sometimes radiating to groin but nowhere else. Is never a sharp pain. For the last six years she has had frequent burning, painful urination. Has also noticed the urine to be a very strong, foul odor. When her bladder is full the pain is worse. Since her trip in April, 1919, she has lost 9 pounds but has no other symptoms. Slightly incontinence on exertion for the last four years. Slightly increased frequency during day. Nocturia 1. No sudden gushes of urine. No edema. Headaches in top of head, usually caused by drinking coffee.

Cardiac respiratory: "Hay fever." Is dyspneic on exertion. Appetite poor since April, 1919. Bowels regular.

Physical examination: Average developed, average sized woman.

Scalp: Negative.

Skin: Negative.

Sclera and mucous membrane: Negative.

Eyes: Negative; pupils, eq. circ., reg., react to light and darkness.

Ears: Negative.

Nose: Negative.

Teeth, gums, tongue, throat, tonsils, neck, thyroid: Negative.

Glands: Occasionally one palpable.

Chest: Average development.

Breasts: Small, very little glandular tissue.

Lungs: Movement normal, resonant throughout. Fremitus negative. Breath sounds normal. No râles.

Heart: Apex in fifth specimen. Normal outline. Sounds regular, fair quality, rather rough and impure. No murmurs. Pulse: eq. reg., normal volume.

Abdomen: Flat, tympanitic, soft. Tenderness over right side. Both kidneys palpable. The right comes way below umbilicus, is freely movable. Left not so easily felt.

Liver: Edge 2 cm. below right costal margin.

Extremities: Negative.

Reflexes:

	R.	*L.*
Patellar	+	+
Achilles	+	+
Babinski	−	−

X-ray: Both kidneys well outlined. No evidence of calculi. Pelvis of left kidney normal in size (fig. 39). Slight blurring of calyces.

Pyelogram: Right kidney, dilated pelvis. Kidney drops ½ vertebrae in standing position, with kink in ureter (fig. 34).

Phthalein: First hour, 35 per cent; second hour, 15 per cent; total, 50 per cent.

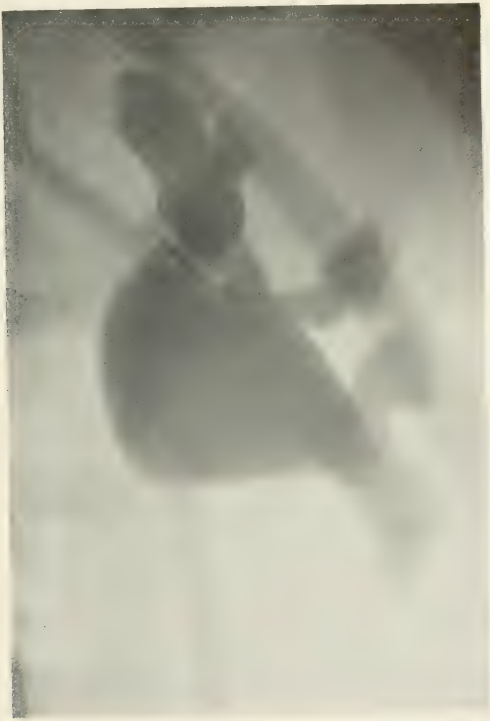


Fig. 34. Pyelogram of the left kidney in case 6, which indicates a kidney not badly damaged yet function was gone and infection pronounced. The actual damage present is shown in figure 35.

January 19, 1920. Patient has been vomiting since her cystoscopy but is feeling very much better today. No vomiting since noon.

January 26, 1920. Patient doing fine. No complaints. Incision clean. Dressing changed.

January 29, 1920. Sutures removed. Incision healing per primum.
February 6, 1920. Patient discharged.

Ureteral catheterization record

January 15, 1920

	RIGHT	LEFT	TRANSVESICAL
Kidney findings:			
Red blood cells	Abundant	0	0
White blood cells	Occ.	Many	4–5 per h.d.
Casts	0	0	0
Epithelial cells	Few	Many	Many
Organisms			
Phthalein:			
Appearance time	3	?	
First fifteen minutes	40	2½	
Second fifteen minutes	15	5	
	—	—	
Total	55	7½	
Leakage	Less than 1		

Ureteral catheterization record

January 17, 1920.
Residual urine: See previous chart.
Bladder capacity: See previous chart.
Bladder findings (described on chart).

	RIGHT	LEFT	TRANSVESICAL
Kidney findings:			
Cultures: Not taken			
Phthalein:			
Appearance time	5 minutes	9 minutes	
First fifteen minutes	35 per cent	Trace	
Second fifteen minutes	15 per cent	2 per cent	
Total	50 per cent	2 per cent	
Leakage			

Pyelography: Right pyelogram taken (1) Prone; (2) Standing.
Pelvic capacity: Right, 20 cc. thorium; left, on pelvis.
Pain: Little.

Remarks: No. 6 catheter passed to both renal pelves with ease.

Operation. Left nephrectomy (January 22).

General condition: Good.

Reason for operation: Exploratory of left kidney.

Operation: Gas and oxygen anesthesia. The field of operation was prepared with alcohol, ether and biniodid. An incision about 10 cm. long was made about 2 cm. below the tenth rib and parallel to it, extending from the costo-vertebral angle toward the umbilicus. The subcuticular tissues and muscles (latissimus dorsi and lateral edge of the erector spinae) were divided through the deep fascia, exposing the perirenal fatty tissue. This was dissected by blunt dissection down to and exposing the kidney on this side. Palpation showed this kidney to be very small and soft. It was uncovered of its perirenal fat and exposed. It was about 5 cm. long and contained a markedly dilated pelvis. The condition was apparently one of slow atrophy from long continued hydronephrosis. Because of the extremely low function on this side and of the useless condition of the organ, a nephrectomy was thought to be the operation of choice. The kidney was dissected free from numerous vascular adhesions, these being clamped and tied in succession. The kidney vessels were cleared and three large Ferguson hemostats were applied near the hilus. The ureter was clamped with a Mayo hemostat about 2 cm. below its pelvis after being first brought up out of the wound by a string tape. The ureter was cut off at its point of clamping, its end cauterized with carbolic and alcohol, the ureter released and retracted along its course. The kidney vessels were next cut at the hilus and the kidney delivered. Suture of the renal vessels was accomplished with no. 2 chromic catgut sutures. The clamps were released one at a time. There was no subsequent bleeding. No drainage was used. The deep fascia was closed with running no. 2 chromic catgut, muscles with running locked no. 2 chromic and the subcuticular tissues with no. 1 plain catgut. Two silkworm gut retention sutures were placed and black silk, locked, was used for the skin.

Condition of patient on leaving operating room: Good.

Specimen consists of left kidney and proximal portion of ureter preserved in 10 per cent formalin, measuring 5.5 cm. in length, 2 cm. in greatest thickness, and 2.6 cm. greatest distance across.

The capsule does not appear thickened. Hyperemic areas show through on its surface corresponding to scar-like depressions where it is adherent. Except for these few adhesions the capsule strips readily.

The kidney appears shrunken, the cortex presents deep irregular depressions from what are apparently old scars, and hyperemic areas varying from pin point to 1 cm. in diameter show through on the cortex.

On section almost the entire parenchyma is seen to be obliterated (fig. 35). The cortex is represented by a thin shell less than 1 mm. thick except one small area about 1 cm. long, where the cortex is about 0.5 cm. thick. This, however, is irregularly hyperemic and the normal structural markings are not clear.

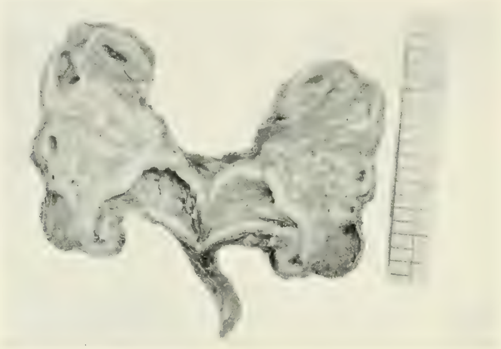


Fig. 35. Left kidney of case 6, after removal pyelogram in figure 34. One small secretory nodule at the lower pole remains. The specimen closely resembles that of dog 17-13, figure 10. There is almost complete atrophy, which hardly can be considered disuse atrophy, as the opposite kidney is movable and hydronephrotic and unable to compensate for the loss of the left kidney.

The pelvis is enlarged, and large dilated calyces have apparently encroached on the kidney substance, running clear out to the cortex, and destroying the whole parenchyma by pressure atrophy. The wall of the pelvis is greatly thickened and thrown into folds, due probably to contracture of a fibrous nature following a primary dilatation. The lobulation on the surface of the kidney corresponds to the dilated calyces within. There is a large amount of fat about the pelvis and calyces.

The ureter appears smaller than normal, its lumen near its proximal end being less than 2 mm. in diameter and its wall slightly thickened and very stiff.

The specimen is unusual in showing almost complete destruction of the parenchyma.

Microscopic section (comprising sagittal section of the whole kidney) reveals an extreme picture of hydronephrotic atrophy. The capsule is very slightly thickened and adherent in a few places. The tubules are atrophied; they possess no lining epithelium and contain hyalin material. The individual tubules appear widely separated due to the extreme fibrosis and lymphocytic infiltration of the interstitial tissue. The blood vessels are thickened. Small foci of lymphocytic infiltration are seen here and there in the neighborhood of the pelvis.

The glomeruli are entirely obliterated except in one small area noted in the gross, where the cortex was about 0.5 cm. in thickness. Here there are a large number of glomeruli, none of which, however, appear normal. The tufts are swollen, some adherent to the capsule; others present hyalin changes and lymphocytes; in many the capsules present fibrous thickening. The tubules here show extreme parenchymatous degeneration. There is peritubular hemorrhages in several areas. The pelvis exhibits submucosa thickening, and exudate of chronic inflammatory type. There is a large amount of fat about the pelvis.

Diagnosis. Hydronephrotic atrophy, irregularly distributed with contraction. Chronic glomerulo nephritis. Evidence of old infection.

Clinical laboratory record

Blood

	WHITE BLOOD CELLS	NEUTRO-PHILES	EOSIN-OPHILES	BASO-PHILES	LYMPHO-CYTES	LARGE MONO-NUCLEARS AND TRANSIT
1/21/20	8,000	68	1	0	22	9

Urine

	AMOUNT	SPECIFIC GRAV-ITY 1000 AND	REAC-TION	ALBU-MIN	SUGAR	
	cc.			*per cent*	*per cent*	
1/15/20	5	18	Ac.	0	0	Few pus cells; many epithelial cells; no red blood cells. Few bacteria
1/19/20	5	14	Ac.	0	0	Few pus cells. No red blood cells; many epithelial cells. Occasional bacteria
1/21/20	5	17	Ac.	0	0	Few pus cells; many epithelial cells

May 7, 1920. Rëentry. For right nephropexy.

Phthalein: First hour, 45 per cent; second hour, 20 per cent; total, 65 per cent.

May 8, 1920. Operation: Right nephropexy.

Operation. General condition: Good.

Reason for operation: Fixation of a ptosed right kidney with starting hydronephrosis.

Operation: Gas and oxygen anesthesia. The field of operation was prepared with alcohol, ether and biniodid. A skin incision of probably some 14 cm. was carried about 1 cm. below the costal margin, the center of the incision being slightly to the rear of the mid-axillary line. This incision was carried directly down through the subcutaneous tissue and muscles to the perirenal fat. Blunt dissection by means of Mayo hemostats produced yellow tinged fat diagnostic of position of the kidney. This was stripped back and the capsule bared. The kidney was then delivered into the wound also by blunt dissection and all adhesions freed. After the ureter was shown to be thoroughly free below, three retaining sutures of Pagenstecher were taken into the kidney substance. Small pads of the perirenal fat which had been removed previously were placed beneath any extra-renal portions of the Pagenstecher suture. These sutures were so placed that one was at the superior pole and slightly anterior to the lateral posterior line; the second was just posterior to the same line in the middle of the kidney, and the third was taken near the lower pole in about the same position as far as the lateral line of the kidney was concerned. All of these sutures were placed some 3 mm. deep. A long curved needle easily brought the superior sutures up under the rib margin and the kidney was held fast there by bringing both ends of the suture upwards and fixing it by a tie. The two lateral sutures were brought through the lumbar muscles, fixing the kidney well laterally by that method. All were tied and cut and the kidney found to be in fixation. Closure was effected by means of two locked chromic no. 2 muscular sutures, doubled no. 00 iodized running suture subcutaneously and plain running silk in the skin. Two deep kangaroo tendon retention sutures were placed and tied.

Condition of patient on leaving operating room: Good.

May 13, 1920. Silk sutures out. Wound in excellent condition.

May 20, 1920. Wound all healed.

May 23, 1920. No complaint of any character. Eating well and gaining weight.

June 2, 1920. *Discharged.* Condition good.

Discussion. Case 6 is an example of bilateral injury with final atrophy on one side and shows the great benefit from repair on the other in response to the definite demand for it. The interesting point for speculation is in regard to the relative duration

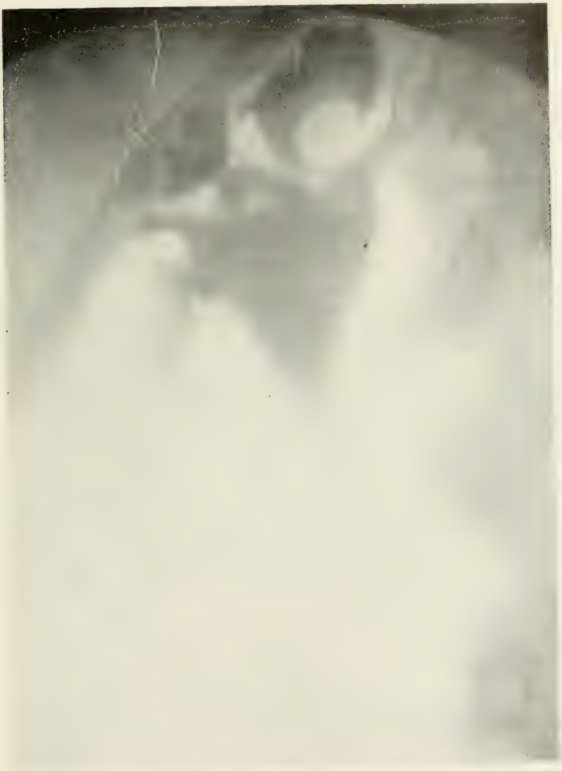


Fig. 36. Pyelogram of right movable kidney of case 6 treated at a second operation (first, left nephrectomy) by uretero-pelvic plastic and fixation. Complete cure, no infection, total function, normal.

of the injury on the right and left sides. A healthy condition on the right would have hastened the atrophy on the left, and it is more than probable that the left hydronephrotic damage has come on after the right function had ceased from an earlier damage. Had they been concurrent the left might not have atrophied so extensively.

Case 7. Girl, age six. Admitted, July 26, 1918.

Complaint. "Thick and shreds in urine."

Family history. Father living and well; mother living and well; two sisters living and well; one brother living; one brother dead (twin and died at birth). No miscarriages. No history of tuberculosis, cancer or epilepsy in family.

Past history. Full term—normal delivery. Breast fed fourteen months.

Development: First tooth at six months; walked at nine months; talked at eighteen months.

Diseases: Chicken-pox and measles.

Accidents: None.

Operations: Tonsillectomy, adenectomy, hemorrhoid.

Past illness. Three years ago mother first noticed urine was cloudy. Before this the child had been in the habit of getting up frequently during night, but for last six months this has diminished, although there has been burning and pain on urination. There seems to be a great deal of thirst and child drinks a large amount of water.

Physical examination. Patient is a fairly well developed but undernourished child of six years.

Skin and mucous membrane seem pale. Heart is not remarkable.

Hair is rather thick.

Eyes, pupils equal and regular and react to light and accommodation.

Nose not remarkable.

Mouth: Tongue clean; teeth well kept. Tonsils out and pharynx clean.

Ears apparently negative. Posterior cervical lymph nodes are the size of peas and hard.

Chest: Rather poorly clothed. Infraclavicular fossae rather marked.

Lungs: Expansion good and equal on the two sides. Resonance good throughout. Tactile frenutus neg. Breath sounds of good quality. No râles heard.

Heart: Outline negative. Sounds of good quality. Rhythm good. No murmurs.

Abdomen: Slightly thin but soft. Edge of liver palpable below costal margin. Tip of spleen felt on deep palpation. No tenderness.

Extremities: Not remarkable.

Skin: There are a few papules and scabs over the flexor surface of the forearms, on one shoulder, on one cheek and on both legs. (The child says her sister has it too.)

Reflexes: Normal.

July 30, 1918. Wassermann taken, negative.

July 31, 1918. Blood pressure: systolic 90; diastolic 60.

August 6, 1918. Catheterized specimen of urine sent to laboratory for G. P. inoculation.

Cystoscopy. Showed large dilated ureteral orifice, with a diffuse granular cystitis. No definite ulcerations seen. No foreign bodies in bladder; no diverticulum. Neither ureteral orifice shows papules or edematous appearance of tuberculosis. Each catheterized. Specimen collected through catheters is same in character as bladder urine. Many pus cells in both; no organisms seen on stained smear. Functional study not done but patient taken to x-ray room and pyelograms (shown in figure 37) showed marked deformity of each pelvis. Major and minor calyces are blunted and dilated and there is considerable cystic formation. Pyelograms indicate a chronic pyelonephritis, with blunting and dilatation of the pelvis and calyces, which in view of the repeated urinary cultures, is probably due to tuberculosis. In view of the bilateral nature of the condition, nothing surgically can be done. Report of guinea pig inoculation never obtained.

April 14, 1920. Patient was returned to the hospital and mother states that she has been fairly well since her discharge two years ago until about three weeks ago, when she fell, striking the abdomen over the bladder. At the next urination she became faint and nauseated and had to be put to bed. After the first two days she was able to get up for a short time but since then, or for the last two weeks, has been continuously in bed. The frequencyand pain at end of urination have been gradually increasing in severity, so that now she voids about every thirty minutes and complains of a severe pain in the bladder region and in the kidneys. The pain comes on each urination and lasts as a rule for about five minutes. She practically always has a bowel movement with each urination during the last two weeks and the mixture sometimes contains blood, which has been thought to come from hemorrhoids. She has no headaches, no frequent coughs or colds. Was gaining in weight until present illness. No night sweats. Appetite good until four days ago, when it was necessary to take laxatives for constipation.

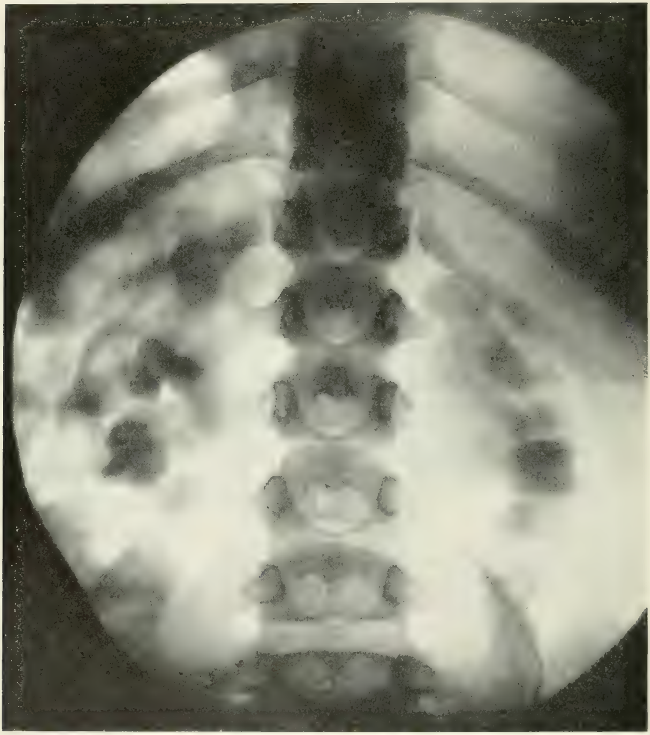


Fig. 37. Bilateral pyelogram showing enormous hydronephrotic sacs on both sides, with tortuous ureters as large as intestines. Marked renal insufficiency and urinary sepsis. Two stage repair operations with remarkable improvement of function and the renal infection (case 7).

Physical examination. Aside from the child being rather thin and pale, the physical examination is negative, as reported in previous history.

April 19, 1920. Phthalein test: First hour, 4 per cent; second hour, 10 per cent; total, 14 per cent.

Ureteral catheterization record

April 15, 1920

	RIGHT	LEFT	TRANSVESICAL
Kidney findings:			
Red blood cells	Not running	1–2 qhd.	2–4 qhd.
White blood cells		1–2 qhd.	Field solid
Casts		0	0
Epithelial cells		0	Occas.
Organisms		0	0
		Mostly mucus	

Cultures: Taken, bladder and left kidney. No bacteria.
Cultures sterile. G. P. inoculation.

	RIGHT	LEFT	TRANSVESICAL
Phthalein:			
Appearance time	Not running	8 minutes	
First fifteen minutes		Slight trace	
Second fifteen minutes			
Total			
Leakage			0 per cent

Ureteral catheterization record

April 20, 1920

	RIGHT	LEFT	TRANSVESICAL
Kidney findings:			
Red blood cells		0	3–4 qhd.
White blood cells		2–4 qhd.	Clumps
Casts		0	0
Epithelial cells		0	Few
Organisms		0	Rods +

Cultures: Bladder, right kidney and left kidney. Bladder: Streptococcus viridans; left kidney, sterile.

	RIGHT	LEFT
Phthalein:		
Appearance time	Not catheterized	9 minutes
First fifteen minutes	Transvesical at end of fifteen minutes	1½ per cent
Second fifteen minutes	Showed only slight trace of phthalein	
Total		
Leakage		

April 23, 1920. Patient much more comfortable. Has no frequency nor pain following urination, as so badly complained of on admission.

A catheter previously placed in the ureter was frequently irrigated with argyrol, and on May 8 phthalein test showed by way of this catheter from left kidney—first hour, 10 per cent; second hour, 20 per cent; whereas by way of the bladder from the right kidney during the first hour only a faint trace was obtained, and in the second hour, 3 per cent.

Operation. (May 1.)

May 1, 1920. Operation: Left ureteropyloroplasty.

General condition: Good.

Reason for operation: Impaired kidney function.

Operation: Gas, oxygen and ether anesthesia. The field of operation was prepared with alcohol, ether and biniodid. An incision was made in the lumbar region just below and parallel to the twelfth rib on the left side. The kidney and its pelvis were delivered. There was very little kidney tissue left, the pelvis being markedly dilated. There were several tubercular nodules palpable in the kidney substance. Lobulation was very marked. There was a small constriction at the pelvic ureteral junction. This was divided transversely, the incision being sewed up so that the long axis of the wound was at right angles to its former position, the same as in a pyloroplasty. No. 00 chromic catgut was used. The ureter itself was the size of a fountain pen and apparently dilated for its entire length, as much foul-smelling urine flowed back from it on section. A small soft catheter was sewed into the ureter for irrigation. Closure was made in the usual manner, a rubber drain being inserted.

Condition of patient on leaving operating room: Good.

May 10, 1920. Stitches removed.

May 13, 1920. The drainage catheter in ureter was removed and the drainage through the lumbar incision gradually diminished, with a corresponding increase in urinary output.

May 15, 1920. Phthalein: First hour (600 cc. output), 10 per cent; second hour (200 cc. output), 15 per cent; total, 25 per cent.

May 18, 1920. Large ureteral catheters were inserted by cystoscope and retained during the next two days. Culture of the urine at this time, for the first time, showed B. coli. The ureteral catheters on each side were lavaged every three hours with 1 per cent mercurochrome.

May 21, 1920. Cultures: From right kidney showed B. proteus; from left kidney and bladder, B. coli, B. proteus, Streptococcus viridans.

May 23, 1920. Clinical condition has improved, evidence of less sepsis.

Phthalein: First hour, 20 per cent; second hour, 15 per cent; total, 35 per cent.

June 1, 1920. Temperature has gone down and clinical sepsis has been reduced. Culture from catheterized specimen of bladder shows B. coli and B. pyocyaneus.

Ureteral catheterization record

May 18, 1920

	RIGHT	LEFT	TRANSVESICAL
Kidney findings:			
Red blood cells	Occas. cell	Occas cell	None
White blood cells	Abundant	Abundant	Abundant
Casts	None	None	None
Epithelial cells	Much detritus 6 h.d.	10–12 h.d.	Debris—12–15 h.d.
Organisms	Rods	Rods	Numerous rods

June 3, 1920. Cystoscopy

Kidney findings:			
Red blood cells	0	0	0
White blood cells	Abundant (300 h.d.)	Abundant (250 h.d.)	200 to h.d. field
Casts			

Remarks: Retention catheter placed in each kidney pelvis.

June 1, 1920. Wound looks better. Anterior portion is about healed.

June 2, 1920. Phthalein: First hour, 30 per cent; second hour, 30 per cent; total, 60 per cent.

Catheters left in place for four days, during which time both kidney pelves were lavaged with mercurochrome every two to four hours.

June 7, 1920. White count, 11,000; 33 per cent small lymphocytes; 2 per cent eosinophiles; 56 per cent polymorphonuclears; 2 per cent fragmentary leucocytes; 4 per cent large lymphocytes; 1 per cent endocytes; 1 per cent Basophiles.

Phthalein: First hour, 30 per cent; second hour, 30 per cent; total, 60 per cent.

Physical examination shows heart and lungs in good condition for anesthetic.

June 11, 1920. Patient has been running a fever since operation but this is going down. Mercurochrome irrigations given through ureteral catheter every three hours. Wound is dry.

Phthalein: By way of bladder: First hour, 7.5 per cent; second hour, 10.0 per cent; total, 17.5 per cent.

Though catheter in right ureter: First hour, colored with mercurochrome; second hour, 2 per cent.

This test would show that the previous operated kidney is doing the major amount of work.

June 15, 1920. Ureteral catheter apparently not draining well. Irrigated and washed out. Wound at left side is a little raw and open.

June 16, 1920. Phthalein: Through ureteral catheter: First hour, 10 per cent; second hour, 15 per cent; total, 25 per cent.

Through bladder: First hour, 7.5 per cent; second hour, lost.

Wound dressed. Catheter removed from right ureter.

June 19, 1920. Wound dressed and remaining silk sutures removed.

July 3, 1920. Phthalein: First hour, 20 per cent; second hour, 10 per cent; total, 30 per cent.

Operation. General condition: Fairly good.

June 8, 1920. Second operation (right ureteropyeloplasty).

Reaon for operation: Hydronephrosis and pyelitis.

Operation: Gas and oxygen anesthesia. The field of operation was prepared with alcohol, ether and biniodid. After having the patient well on her left side and in good position so as to give a good exposure for a kidney incision, the usual incision was made just below and parallel to the twelfth rib on the right side. The kidney with its pelvis and ureter was then fairly easily exposed and delivered. The kidney was found to be fairly well collapsed. The ureter was dilated about 0.75 cm. down as far as could be seen or felt. There was found to be a kink or constriction of the ureter at the uretero-pelvic junction. All the adhesions between the ureter and pelvis and the ureter and kidney were freed and a longitudinal incision made through the kinked area, which was sewed up so as to make the long axis at right angles to the line of incision, thus widening out the constriction. A small rubber catheter was then inserted down deep into the ureter through a previously made incision in the pelvis. A rubber tube and rubber dam drain were placed in the wound and the muscles and fascia closed with continuous no. 2 iodized catgut. The skin was closed with buttonhole silk sutures. Dry dressings applied.

Condition of patient on leaving operating room: Good.

July 8, 1920. During the last few days temperature has been up; highest, 39.9°. Patient feels fine other than loss of appetite. No pain.

Physical examination. Chest negative; respirations rapid, 26 per minute; heart negative, heart sounds negative; rate, 128 per minute; no murmurs. No tenderness or pain in abdomen.

Temperature gradually subsided within next few days.

July 11, 1920. Patient discharged. Temperature 37.2°.

August 9, 1920. Reëntry.

Past illness. Since discharged on July 11, 1920, the patient was doing better; gained 7 pounds during three weeks' time. Drainage from the wound was stopped. Voided about 40 ounces of urine a day, more or less clear. No pain in bladder or kidney region. However, between July 31 and August 5, she had fever (101); nausea and vomiting; was unable to retain food. Amount of urine decreased to 30 ounces and was more cloudy and had foul odor. Had pain over the umbilical region about half hour before the urination. These troubles had taken place just during those five days (July 31 to August 4). She has been well rest of days while at home.

Physical examination. Is essentially negative except that she is delicate, pale, anemic and highly nervous. General condition, however, is very much better now than when she left the hospital three weeks ago.

August 9, 1920. Phthalein: First hour, 25 per cent; second hour, 25 per cent; total, 50 per cent.

Urine: Cloudy, greenish gray. Specific gravity 1.006; alkaline albumen 0; sugar 0; abundant white blood cells; red cells 0; many rods and cocci.

Culture: Bladder, Streptococcus viridans; left kidney, Streptococcus viridans.

August 10, 1920. Cystoscoped. Catheter inserted into left kidney, which was retained for six hours, during which time three irrigations with 5 cc. of ½ per cent mercurochrome were given.

August 12, 1920. Right kidney catheterized and lavaged every three hours for a period of twelve hours, with 1 per cent mercurochrome.

August 14, 1920. Discharged.

August 17, 1920. Reëntry for observation.

August 18, 1920. Phthalein: First hour, 20 per cent; second hour, 10 per cent; total, 40 per cent.

Urine: Cloudy, yellow; alkaline albumen 0; sugar 0; casts 0; white blood cells abundant; red blood cells 0; abundant cocci and rods present.

August 19, 1920. Cystoscoped. Catheter introduced into left ureter. Mercurochrome instillation every three hours via catheter.

Culture: Streptococcus viridans.

August 22, 1920. Blood count: Hemoglobin 60 per cent; red blood cells 4,560,000; white blood cells 14,200; polymorphonuclears 64 per cent; S. M. 25 per cent; L. M. 9 per cent; eosinophiles 2 per cent.

August 22, 1920. Patient feeling well. Temperature almost normal since entry. General appearance good.

August 23, 1920. Patient discharged.

December 1, 1920. Reëntry for examination. Since last entry patient has gained 5 pounds. If she gets over-tired she complains of pain in pit of stomach and is nauseated. Usually gets up only once during the night to void. No hematuria; no burning on urination. Appetite good. Good control of urination. Bowels still constipated and patient continues with cathartics. Sleeps good. Much more active than ever before and does not tire nearly so easily. Lungs negative; heart, normal area of cardiac dullness; sounds of good quality and rhythm.

December 3, 1920. Phthalein: First hour, 20 per cent; second hour, 15 per cent; total, 35 per cent.

Cultures of catheterized urine show B. coli in all three specimens.

April 14, 1922. Patient has been recently heard from and has remained about the same as indicated by last note. No further cystoscopic studies or phthalein tests have been done.

Case 8. Female, age fifty. Admitted, August 16, 1921.

Complaint. Frequency, pyuria, night sweats.

Family history. No tuberculosis malignancy, mental disease. No renal disease except as terminal event.

Past history. Recently married. No children. Cta: Thirteen years 28/4, no clots, pain or leucorrhea.

Diseases: Measles, mumps, chicken-pox. No infectious diseases; no scarlet fever, diphtheria or pneumonia. Occasional sore throats and tonsillitis formerly. No C. R. history. Appetite good, weight maintained. Is constipated; taken no laxatives. Complains of dull oppressive feeling in epigastrium during past year, unassociated with eating, position. Not noticed as much when eating "light" food. No other G. S. history. Sleeps fairly well but of nervous disposition.

Past illness. Accidents: None.

Operations: Kidney fixation 1903, Portland, Oregon. Following operation, patient told that there was a "kink" in right ureter. Patient had no genito-urinary symptoms prior to kidney fixation, but mobile kidney on incidental finding in physical examination at that time. Four weeks following operation developed cystitis with burning, frequency and suprapubic tenderness. Treated at time with boric irrigations, argyrol and $AgNO_3$, and continuously for nine months. Following discharge had no further vesical symptoms or genito-urinary disturbance until onset of present illness. About June 10, patient noticed slight night sweats and a few days later began to have severe "urethral pain—not burning" at the end of urination. This became so severe patient called physician. She was taken to Ward Sanitorium and treated for B. coli pyelitis with vaccine and bladder irrigation. Following discharge in three weeks subjectively well until August 7, when patient experienced dull ache and throbbing over left lumbar region posteriorly. This lasted twenty-four hours and patient called physician, who gave hyperdermic. Kept on liquid diet in bed; felt well for a week and had a similar attack, lasting twenty-four hours. Given another hyperdermic, which did not relieve all pain. Following this patient had slight burning on urination. Noticed no frequency or other urinary disturbance. During both attacks patient vomited once. In interim had some fever and slight headache. No chills. Dr. Hinman called and patient entered hospital.

Physical examination. Fairly well developed and nerved woman. General appearance in keeping with age.

Skin clear. Right rectus scar and scar over right back.

Eyes, ears, nose: Externally negative.

Mouth, tongue and throat: Negative.

Chest: Walls symmetrical; equal movement and respiration.

Lungs: Resonant; no rales or changed breathing heard.

Heart: A. C. D. of usual dimensions; sounds regular in rate, force and rhythm but distant and feeble. No murmurs. Pulse (right) equal, synchronous, regular in rate, force and rhythm. Volume good and tension moderate. Blood pressure 120/80.

Abdomen: Symmetrical. No local prominence. Tenderness on deep palpation over left kidney. No tenderness over right on gentle palpation. No edema, induration, inflammation or neighborhood fluid.

Back: Scar as mentioned. No tenderness.

Extremities: Negative.

Reflexes: Arm and leg jerks of usual amplitude and equal. No pathological spinal tendon jerks elicited.

August 16, 1921. Patient to cystoscopy. Ureters catheterized. Pelves of kidneys drained. Both specimens exhibited marked pus content. Five cubic centimeters 0.5 per cent $AgNO_3$ injected into pelvis of right kidney and the left kidney catheter allowed to remain in place.

Phthalein:

	R	*L*
Appearance time	18	25
First fifteen minutes	6	Trace
Second fifteen minutes	Not collected	

August 17, 1921. Patient had good night. Catheter draining well. Night specimen about 20 per cent pus. Patient nearly afebrile today and fluids well taken. Subjectively well.

Culture urine both kidneys catheterized—B. coli. Vaccine 1 billion to cubic centimeter to be made.

August 18, 1921. Temperature, pulse and respiration normal this evening, though patient complains of pain over right kidney and is restless. Catheter removed following lavage this afternoon.

To cystoscopy. Kidneys catheterized to pelves; marked pyuria of right side; no drainage from left.

Blood: Urea nitrogen, 19.0 mgm. per 100 cc.; non-protein nitrogen, 40.0 mgm. per 100 cc.

August 19, 1921. Patient resting well.

Urine: 1.009, epithelial cells many; red blood cells, 0; white blood cells 50–60 h.d.; casts 0.

Phthalein: First hour, trace; second hour, 12 per cent.

August 21, 1921. Urine: Gross specimen shows great improvement; is cloudy with only about 5 per cent sediment. Specific gravity 1.01; acid; white blood cells 50 h.d.; red blood cells 0; casts 0.

August 30, 1921. Patient has been experiencing severe and aching pain over left kidney region, yesterday and today, with definite local tenderness anteriorly and posteriorly.

To cystoscopy. Both ureters catheterized. Only 2 cc. slightly cloudy urine from left side, with no subsequent drainage, so catheter withdrawn. Right catheter retained and lavaged with 1 per cent mercurochrome during morning and afternoon every three hours. Patient in considerable distress because of pain.

August 31, 1921. Patient nauseated during day. Has taken very little food. Output yesterday, 1800. Pain on left side absent today and patient more comfortable.

Phthalein: First hour, trace (about 10 cc. urine only in specimen); second hour, 5 per cent (very little urine—about 20 cc.).

September 2, 1921. Patient complains of slight urethral irritability today.

Phthalein: First hour, 15 per cent; second hour, 20 per cent; total, 35 per cent.

September 3, 1921. To cystoscopy. Right kidney pelvis lavaged with 1 per cent mercurochrome and repeated every three hours during day. Catheter withdrawn at 7:00 p.m.

September 6, 1921. No backache or renal pain during past three days. Sat up for fifteen minutes yesterday.

Urine specimens still very cloudy but show diminishing amount of sediment. Centrifuged specimen gives about 1/20 sediment.

Phthalein: First hour, 15 per cent; second hour, 20 per cent; total, 35 per cent.

September 7, 1921. Urine: Hemogeneous sediment about 1/20. Uncentrifuged specimen shows: White blood cells 2–4 h.d.; red blood cells 0; casts 0; epithelial occasional round cell.

September 9, 1921. To cystoscopy yesterday. Right kidney pelvis lavaged with mercurochrome. Catheter retained and lavaged every three hours during day. Urine shows considerable improvement over previous catheterized specimen.

September 12, 1921. Patient improved. Urinary output averages 1800. Specimens macroscopically show fine turbidity but greatly reduced amount of sediment, being 1/40 or less.

Urine in uncentrifuged specimen shows only occasional white blood cells in contrast to previous findings. Centrifugalized specimen shows field covered with white blood cells, epithelial cells and motile rods.

September 13, 1921. To cystoscopy this morning.

Ureteral catheterization record

September 13, 1921

	RIGHT	LEFT	TRANSVESICAL
Kidney findings:			
Size of catheter			No specimen
Distance inserted	Pelvis	Pelvis	
Character of flow	Continued	Continued	
Uncentrifuged:			
Macroscopic			
Microscopic			
Red blood cells	0	0	
White blood cells	6–8 h.d.	6–8 h.d.	
Casts	0	0	
Epithelial cells	Occas.	Occas.	
Organisms	Motile rods	Motile rods	
Stained smear			
Cultures: Right and left kidney, bladder			
Phthalein:			
Appearance time	8	8	
First fifteen minutes	5	Trace	1st hour, 20
Second twelve minutes	17	7	2nd hour, 15
			—
Total	22	8	35
Leakage	Not collected		

Remarks: Both ureters catheterized and lavaged every three hours during day with 1 per cent mercurochrome.

September 13, 1921. Left pyelogram was made with sodium bromide, which is in every way similar with that reported of the right kidney (fig. 43).

September 14, 1921. Temperature, pulse and respiration normal. Patient fairly comfortable during early part of day. Experienced several short attacks of severe cramp-like pain along course of both ureters. Urinary output remains fairly high and character as previously noted. Slight turbidity with very little sediment now. Microscopic examination shows field covered with pus, cells and rods.

Phthalein: First hour, tinged with Hg 220 and phthalein precipitated (color of sediment indicates considerable phthalein present); second hour, 20 to 30 per cent, tinged slightly with Hg 220.

September 16, 1921. Phthalein: First hour, 10 per cent; second hour, 40 per cent; total, 50 per cent.

September 19, 1921. Phthalein: First hour, 25 per cent; second hour, 30 per cent; total, 55 per cent.

Culture: (September 15), B. coli in all three.

Urine: Slightly cloudy; albumen S. P. T.; white blood cells 40–50 h.d.

September 21, 1921. Urine culture: (January 17), B. coli.

Phthalein: First hour, 10 per cent; second hour, 30 per cent; total, 40 per cent.

September 23, 1921. Phthalein: First hour, 15 per cent; second hour, 20 per cent; total, 35 per cent.

Urine: Cloudy; albumen trace. Microscopic examination, white blood cells 15–20 h.d.; many motile rods.

Bladder urine culture: B. coli.

September 24, 1921. Right pyelogram, as shown in figure 38, was taken with sodium bromide 20 per cent. The enormous hydronephrotic sac is quite evident.

September 30, 1921. Phthalein: First hour, 20 per cent; second hour, 30 per cent; total, 50 per cent.

Urine culture from right and left kidney and bladder showed B. coli.

October 5, 1921. Temperature this morning, 37.7; pulse, 102.

(October 3.)

Reason for operation: Hydronephrosis and nephroptosis.

Operation. Pyeloplasty and nephropexy.

October 3, 1921. Operation, uretero-pyeloplasty and nephropexy.

Patient was placed on her left side.

Skin cleaned with alcohol, ether and biniodid.

An incision was made parallel to and about 1 cm. below the left rib, extending from the subcostal angle behind and completely downward to the anterior iliac spine. The muscle layers were divided in the usual way and the perinephric fat identified. This was excised and the kidney exposed. A large hydronephrotic kidney was seen similar in outline in all respects to the x-ray plates. There was a very large extra-renal urine-filled pelvis and an elongated and broadened parenchyma. To the palpating finger the parenchyma seemed fully 3 cm. thick. The kidney was readily freed and the ureter identified and separated. A retaining tape was passed around it. The ureter was not greatly enlarged or thickened but its course ran obliquely forward from the posterior inferior aspect of the hydronephrotic sac. On reaching the anterior aspect of this sac the direction of the ureter changed abruptly downward toward the bladder. There was passed to the operator a no. 00 black silk suture on a curved French needle. Beginning at the upper pole of the kidney at the junction of the pelvis and renal tissue proper this silk suture was placed in the outer fibrous coat of the pelvis at $\frac{1}{2}$ cm. intervals, passing from one side of the kidney pelvis around the belly of the pelvis to the other side and back again. A second suture identical with this was placed toward the lower pole of the kidney just beyond the midline. These two sutures were then

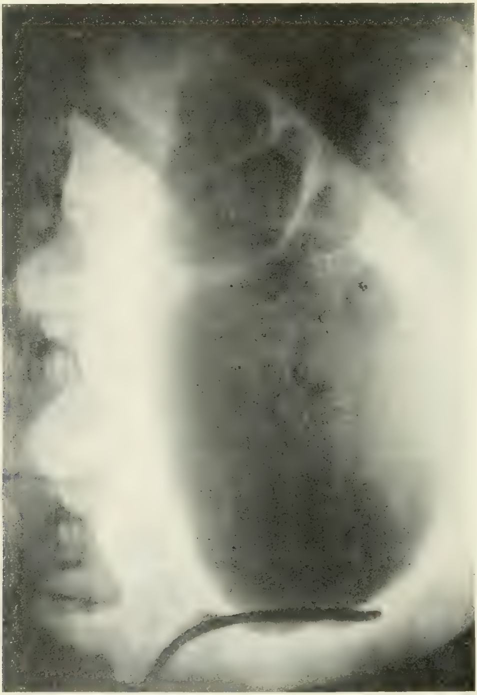


Fig. 38. Case 8. Left pyelogram (the right was in every way similar) showing huge bilateral hydronephrosis relieved in two stages by plastic operation and kidney fixation, with a more gratifying and remarkable result even than in case 7. Total function restored to normal and infection relieved.

drawn tightly and firmly tied. The knots were on opposite sides of the kidney pelvis. The result of drawing in these black threads was to cause an enfolding and tightening of the pelvis, reducing this pelvis space and permitting the free exit of urine down the ureter. With warm tape pads the kidney was now lifted up into the wound. Three sutures of braided silk were placed in the kidney substance with a large curved round needle running close to the capsule in mattress fashion. One of these was placed on each lateral aspect of the kidney in its lower pole and one in its upper pole on its convex border. They were then anchored deeply in the neighboring muscles, the one from the upper convex portion of the kidney being carried through the tissues between the eleventh and twelfth ribs. The deep muscles were then approximated with running locked sutures of no. 2 chromic gut. A second running suture of doubled no. 2 chromic was used to close the superficial muscles. Skin was closed with running locked sutures of black silk. Iodin was applied and the wound covered with gauze dressings. A small drain was allowed to protrude from the posterior edge of the wound.

Patient left the table in good condition.

October 5, 1921. Last night patient given pituitrin and abdominal massage with warm oil for gaseous distension, with considerable relief. Distension returned somewhat and was greatly relieved at 3 a.m. by milk and molasses enema. This morning distension much less than yesterday. Able to retain egg and toast.

October 15, 1921. Phthalein: First hour, 25 per cent; second hour, 30 per cent; total, 55 per cent.

October 19, 1921. Phthalein: First hour, 24 per cent; second hour, 30 per cent; total, 54 per cent.

October 26, 1921. Patient has not been feeling so well last few days. Urine is quite turbid, with heavy white sediment.

October 30, 1921. Phthalein: First hour, 17 per cent; second hour, 21 per cent; total, 38 per cent.

Patient allowed to take a few steps. Up in chair for part of an hour. Feeling well. All urine specimens show heavy mucous sediment; much pus.

November 18, 1921. Patient has gradually been getting stronger. Urine quite cloudy, showing pus.

Phthalein: First hour, 16 per cent; second hour, 33 per cent; total, 49 per cent.

November 19, 1921. Patient discharged.

January 23, 1922. Reëntry. During interval since last discharge patient has been under observation and has had several cystoscopic examinations, with ureteral catheterization and pelvic lavage. Her general health has been fair. Has gained thirteen pounds; walks about a little. Occasional dull pain in either loin and has light night sweats if she has overdone.

January 23, 1922. Phthalein: First hour, 23 per cent; second hour, 28 per cent; total, 51 per cent.

January 24, 1922. *Examination.* Lungs: Negative. Normal resonance, breath and voice sounds. No râles.

Heart: No symptoms. Sounds of good quality. Rate 84 per minute. Blood pressure: 130/82.

January 25, 1922. *Operation.* Right uretero-pyeloplasty and nephropexy.

Reason for operation: Infected hydronephrosis of right kidney.

General condition: Good.

Anesthesia: Gas and oxygen.

The patient was placed in the left lateral position with lumbar support. An incision was made parallel to and about 1 cm. below the twelfth rib in the region of a former scar of the same length, which ran somewhat transversely to the present incision. The cut was carried down to and through the muscles with a knife but was carried to the retro-renal region with scissors. The vessels were ligated with no. 00 iodized catgut and towels anchored to the wound edges. A number of adhesions were found comprising the retro-renal tissues. A small amount of retro-renal fat was seen as the fibrous tissue was separated. With warm tape pads the kidney was lifted up into the wound. A $\frac{1}{2}$-inch gauze tape was passed beneath the ureter and grasped with a Kelly hemostat. The kidney was freed from the adhesions on all sides and there was found present a greatly dilated pelvis, which was extrarenal in type. The pelvis seemed to be divided extrarenally to the parenchyma into their limbs. A no. 10 soft rubber catheter was inserted through a stab wound into the pelvis. The contents of the pelvis were withdrawn through this catheter and were found to be full of flakey, blackish mucus. The catheter was then carried down to the ureteral pelvic juncture and passed 1 or 2 cm. into the ureter. A purse-string suture of black silk was carried around the kidney, threading itself just below the superficial layers or adventitia of the kidney pelvis. A second suture of this type was placed nearer the ureteral outlet and the two were drawn up so as to greatly narrow the pelvis. A longitudinal slit was made at the ureteral pelvic junction and was closed transversely with an over-and-over running suture of no. 00 plain catgut. The kidney was then lifted up into the renal fossa, the $\frac{1}{2}$-inch tape withdrawn from around the ureter and the kidney was fixed in its bed by means of three Pagenstecher sutures. The upper of these was placed in the upper pole of the kidney on the convex border; the others were placed in each lateral aspect of the kidney. They were anchored into the surrounding musculature, the upper one being placed between the two last ribs. A rubber drain was placed deep into the kidney fossa, with its lower end at the utero-pelvic junction. The wound was closed in two layers with no. 2 chromic catgut and with several subcutaneous retention sutures of no. 1 catgut. The skin was closed with black silk. The drain was allowed to protrude from the posterior angle of the wound. Gauze dressings were applied.

Patient left the table in good condition.

No specimen.

January 26, 1922. Patient has had little discomfort since operation. Catheterized once at 8:30 p.m. but has voided well since. Slight attack of gas pains relieved by eserine.

January 28, 1922. Condition good. Patient on whole comfortable. Irrigation through ureteral tube being kept up every three hours.

January 31, 1922. Sutures and drainage tube removed. Wound in good condition.

February 7, 1922. Incision has been closing by granulation. Still a small amount of sero-sanguineous discharge from middle and lower thirds and some slight leakage. Ureteral catheter placed well in ureter in an attempt to close the urinary fistula from right kidney. Catheter not open and could not be used as drain, so quickly withdrawn.

February 12, 1922. Eighteen days post-operative. No complaints. Urine output increased February 9 and drainage from wound has been gradually decreasing since. Output past twenty-four hours is 2500 cc., intake 2700. Very little drainage from fistula.

February 13, 1922. Phthalein: First hour, 15 per cent; second hour, 25 per cent; total, 40 per cent.

February 14, 1922. Urinary fistula has remained closed. No urinary leakage for two days.

February 20, 1922. Patient feeling well. General condition improving.

February 28, 1922. Phthalein: First hour, 30 per cent; second hour, 25 per cent; total, 55 per cent.

March 4, 1922. Patient discharged.

April 18, 1922. Since discharge patient has been seen several times and just recently has been cystoscoped and both ureters catheterized. The urine has completely cleared macroscopically but shows few pus cells microscopically and is positive on cultural study for B. coli. Patient's general condition is satisfactory; much stronger and has been running no temperature for months. Function remains between 50 and 60; and the right kidney at this time seems to have a somewhat greater output of phthalein than the left.

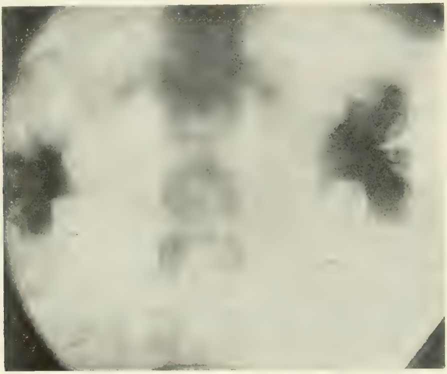


Fig. 39. Plain x-ray plate of a case, showing large stone casts of each pelvis. Pyuria for twenty years, with final marked renal insufficiency. Stones successfully removed by two separate operations. Renal function returned to normal and has remained so for four years.

Discussion. Cases 7 and 8 illustrate the remarkable possibilities of repair in hydronephrosis when there is a bilateral and even demand (also fig. 39). The different functional counterbalances that follow the respective repair operations is striking and give emphasis in addition to that of cases 4 and 5 of the danger of delay in the second operation.

VI. SUMMARY

Renal compensation has an important clinical significance and should be considered from an anatomical as well as functional standpoint. Functional counterbalance has reference mainly to total function and in this limited sense counterbalance is complete whenever renal function is normal. But the anatomical changes may not be complete for many months or years later and therefore the process of an anatomical counterbalance has reference to the relative ability of a right and left kidney rather than to total function. Functional tests are of particular value as indication of relative strength but they do not permit an accurate anatomical correlation and therefore anatomical counterbalance is difficult of clinical application. The anatomical changes are gradual and progressive and cannot be definitely measured by relative function. Such tests, however, in conjunction with a knowledge of the pathology present in each kidney, help one to a fairly clear clinical inference of the compensatory changes and their present and potential extent. The end result, when the anatomical counterbalance may be said to have reached a stationary equilibrium, is primarily dependent upon the relative native or potential reserve strength of the two kidneys. When this is qualitatively equal an even or coöperative type of counterbalance results, but when unequal the nature of the competition created thereby causes growth by compensatory hypertrophy in response to increased functional activity or degeneration by disuse atrophy secondary to diminished or lost function.

Complete compensation in an anatomical sense may be used to define an unilateral compensatory hypertrophy of a functional degree and ability equal to that of two normal and healthy kidneys.

Partial compensation may be used to refer to the anatomical condition of a beginning but incomplete unilateral compensatory change and may occur under three circumstances: First, insufficient time for the development of a complete hypertrophy; second, no need for a complete compensation in view of considerable work still being performed by the other kidney; and third, inhibition of hypertrophy, or, rather, of an increase of function that would lead to hypertrophy, by the injurious action of the disease in it or in the opposite side.

What may be called renal decompensation or failure of a counterbalance will be indicated functionally by renal insufficiency and implies anatomically a condition in which compensatory changes cannot occur on account of the fact that renal tissue is already working to the height of its ability. These various considerations may be diagrammatically represented, as in table 2.

The surgical significance of the above experimental and clinical consideration would seem to be two-fold, in accordance to whether the surgeon is dealing with a unilateral or bilateral lesion, and may be summarized as follows:

1. It is useless to attempt preservation or repair of an unilateral injury in face of a healthy and complete compensatory hypertrophy on the opposite side. Therefore attempts to repair must be made before anatomical compensation is complete, and in distinctly unilateral lesions the outcome is always questionable because even a partial compensation of a healthy kidney may in time become complete through the competitive elimination of its less efficient repair mate. It is only in those conditions where the competition between the two sides is equalized qualitatively by the conservative repair procedure on the diseased side that a coöperative counterbalance can be expected.

2. In bilateral conditions conservation of renal tissue should always be given the greatest consideration. Conservative surgery in these cases will often give wonderful results. But in the event of a two-stage operative procedure, each kidney being treated at separate operations, which is almost always required, the second operation must not be too long delayed, because by the first one the previous equilibrium of competition has been overthrown and the repair on the side first operated may be so stimulated and efficient as to render the work of the unoperated and still inefficient side unnecessary. Delay will lead to its final atrophy from disuse.

3. In such a case of bilateral injury with a successful unilateral repair but in which there has been delay in the second operation, the problem may now closely simulate that of unilateral injury with opposite compensatory hypertrophy, and in place of a conservative procedure as originally planned, radical nephrectomy may be to the best interest of the patient.

TABLE 2

Native reserve with potential ability to do more work		Renal tissue	Reserve power absent, working to height of ability already		
A	*B*		*D*		*E*
Renal hypertrophy	Inhibition of hypertrophy	Demand for more work arises	Unable to hypertrophy	or	Has reached maximum stage of hypertrophy
Partial or complete compensation with development of an acquired reserve	Renal insufficiency	Result	Insufficiency		Insufficiency
	Remove inhibiting factor				
	C				
	Compensation by hypertrophy				
		Competition in response to the demand for more work or as result of surplus of supply			

A with A or with C = Coöperative counterbalance.

A with B, D or E = Partial counterbalance of A, which in case of its marked superiority may lead to a complete compensation of A and a gradual disuse atrophy of B, D or E.

B with B = The anatomical change will be relative to the respective value and strength of each.

C with B = Same as A with B.

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Reprinted from The Journal of Urology
Vol. IX, No. 5, May, 1923

EXPERIMENTAL HYDRONEPHROSIS

THE FAILURE OF DIURESIS TO AFFECT ITS RATE OF DEVELOPMENT[1]

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Received for publication August 25, 1922

INTRODUCTION

The mechanism of hydronephrosis is unknown. It is inseparable from the problem of renal activity, and although many physiologists have attacked the latter through an experimental study of hydronephrosis, definite proof of the causes of the many interesting variations met with is still wanting. Brief mention of the many factors that might be expected to influence this mechanism will be left for the discussion. The purpose of this paper is the presentation of an experimental study of the influence of one of these possible factors, namely the rate of urinary excretion, upon the development of hydronephrosis.

The causes of an increase in the flow of urine are at best poorly understood. Renal diuresis has always been the stumbling-block of the many theories advanced in explanation of renal activity. The action of diuresis upon ureteral pressure is uncertain. A constant increase of the ureteral pressure would surely be expected to have an hastening effect upon hydronephrosis. Profuse diuresis from salines and urea have been observed by Cushny (8) and others occasionally to raise the maximal ureteral pressure to 130 mm. of mercury in one dog (Obniski (9)), when the average is about 50 to 70 mm. But all diuretics do not so act; neither do these two thus act uniformly. There are many technical difficulties and variations, too many to be discussed here. Irrespective of the theory in explanation of urinary secretion, can one look for an effect upon the development of hydronephrosis in those conditions influencing more or less continuously the amount of urine excreted. Or will certain types of diuresis only, as, for example, those following an increase of blood pressure, have an influence? There is a certain definite relation between the blood pressure and the ureteral pressure and here the kidney differs from secretory glands, in which secretory pressure is largely independent of blood pressure. But if it is conceivable that renal activity continues in a kidney of which the ureter is completely obstructed—and the formation of large fluid sacs would seem only thus possible—it would seem reasonable to expect that the production of greater activity through the use of diuretics or of relative inactivity through water starvation would result in an alteration of the usual picture.

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[1] Read April 21, 1922, at Atlantic City, before the American Urological Association.

Certain difficulties are immediately presented in contemplation of the investigation, the two greatest of which are the great variation and uncertainty in the mode of action of diuretics in general and the difficulty, in view of our ignorance of its mechanism, of having a proper control of simple hydronephrosis for comparison.

Previous experimental work with large white rats has given us entire confidence in the uniform and progressive development of hydronephrosis with complete ureteral obstruction. Partial obstruction would not be possible of accurate control in any animal and complete obstruction in larger animals gives variations and occasionally, as in the cat or dog, leads to primary atrophy. We have now doubly tied and divided the ureter in over 300 rats and have yet to see the occurrence of a primary atrophy. In these animals the degree of hydronephrosis is uniformly proportional, grossly, to the duration of the obstruction relative to the age and weight inequalities. Microscopically slight differences are due to the impossibility of having for comparison sections from exactly similar portions of the hydronephrotic kidneys. The degree of hydronephrosis of each control animal is readily classified in conformity to the period of the obstruction, and there is no overlapping nor exceptions. The four-day periods may differ somewhat among themselves but differ entirely from the seven-day kidneys, and the seven from the fourteen, and so on. As a group each period is distinct. The rat from the standpoint of control is therefore ideal for the experimental testing of the action of diuresis upon the development of hydronephrosis.

Its choice, however, was unfortunate in that practically no work has been done on this animal with the usual diuretics. Standardization of their action in so small an animal would be tedious and difficult and has not been attempted. For this reason we have chosen a rather complete variety of diuretics and have tried to give a maximal dose of each. On this account our mortality under diuresis, particularly with digitalis and diuretin, has been high, but in order not to vitiate our results only those specimens of animals in good health when sacrificed at the desired period have been used. By observation we know that an enormous increase in the urine output has been kept up daily for the periods desired. Daily intraperitoneal injection of 25 cc. of fluid, more than one-tenth of the body weight, would alone insure this. For purposes of comparison the animals were run in the following series:

1. Control series; free access to food and water.
2. Fluid limitation series; free access to dry food but water given in minimal quantities in moist bread.
3. Urea diuresis series; free access to food and water and the daily intraperitoneal injections of 25 cc. of a 1 per cent urea solution (0.25 gram urea).
4. Salt diuresis series; free access to food and water and the daily intraperitoneal injections of 25 cc. of a 2 per cent solution of sodium chloride (0.5 gram NaCl).
5. Diuretin diuresis series; free access to food and water and daily intraperitoneal injections of 25 cc. of a 0.2 per cent solution of diuretin (0.05 gram diuretin).
6. Digitalis diuresis series; free access to food and water and the intraperitoneal injection of 4.5 cc. of digitalis infusion in 25 cc. of water on alternate days. (Fresh infusion made every third day (Eggleston) by taking 15 grams of the dried leaves and pouring over them 1000 cc. of boiling water.)

EXPERIMENTAL DATA

Procedure

Large white rats of uniform age and weight (average, 200 grams) have been used.[2] Under ether narcosis and surgical asepsis the left ureter was doubly ligated with silk ligatures, just above the bladder, and divided between them through a midline suprapubic incision. The operated animals were carefully labeled by the ear-punch method and segregated for study into the 6 groups mentioned above.

I. The Control Series

The characteristically uniform increase in size of hydronephrosis in the rat with unilateral ureteral obstruction is demonstrated in table 1. The measurements of length, width and depth were made with a micrometer, as shown in figure 1. There is a marked slowing of the rate of increase by the end of the sixth week—42 days (see also fig. 2), but enlargement continues up to 365 days, when the sac holds about 12 cc. of smoky fluid, usually containing many red blood cells. We have no observa-

TABLE 1

Measurements showing uniform increase in size of hydronephrotic kidney in rats after total ligation of left ureter

DURATION	NUMBER OF RATS REPRESENTED	AVERAGE WEIGHT OF RATS	AVERAGE KIDNEY MEASUREMENTS		
			Length	Width	Depth.
days		*grams*	*cm.*	*cm.*	*cm.*
0	30	194	1.63	0.82	1.00
4	9	210	1.84	1.02	1.04
8	14	214	2.15	1.12	1.15
14	20	229	2.35	1.50	1.31
28	21	243	2.55	1.68	1.43
42	17	235	2.72	1.78	1.64
56	14	230	2.71	1.92	1.71
365	5	260	3.51	2.35	2.16

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[2] We are indebted to Dr. Herbert C. Evans for his generosity in furnishing us with young adult and healthy rats of uniform breed.

tions of longer duration than one year, but further enlargement would not seem possible in view of the mere connective tissue sac then present. Similar weight curves of hydronephrosis in rabbits, published by Holste (10), Ponfick (11), and Winkler (12), ran closely parallel to these for rats. The uniformly progressive nature of the hydronephrotic atrophy is well demon-

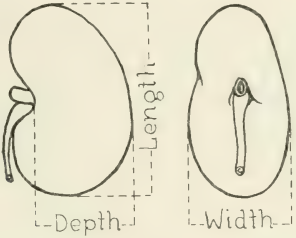

Fig. 1. Diagram to Show Method of Measurement of Kidneys for Depth, Length and Width

In measuring the depth extra-renal pelvic dilatation is disregarded.

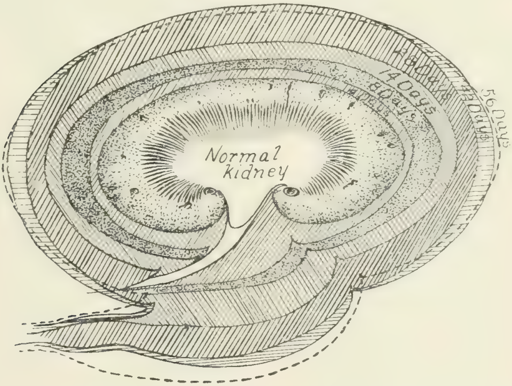

Fig. 2. Composite Drawing to Represent the Progressive Increase in Size of the Kidney for the Different Periods of Complete Ureteral Obstruction

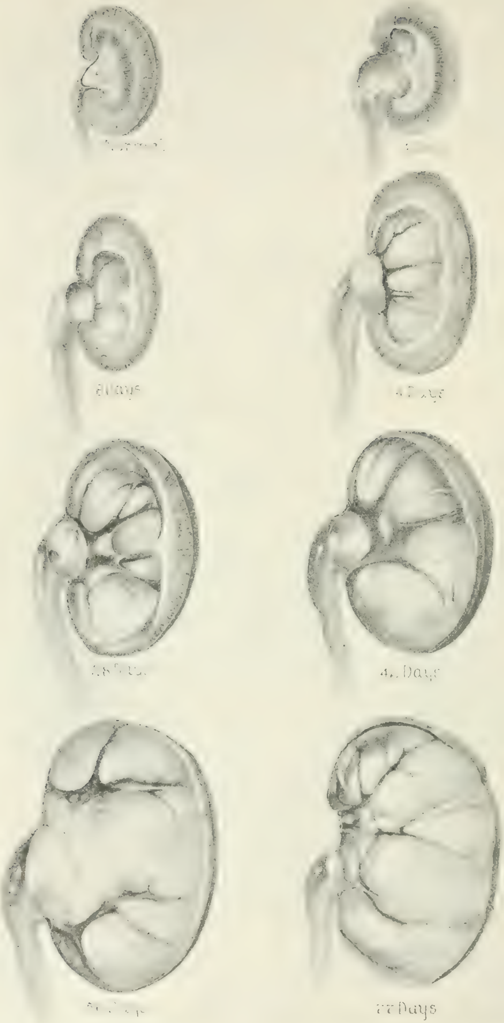

FIG. 3. DRAWINGS OF REPRESENTATIVE HYDRONEPHROTIC RAT KIDNEYS CUT SAGITALLY FOR THE DIFFERENT PERIODS OF OBSTRUCTION

Compare with the drawings of similar specimens but cut in cross and coronal section, shown in figures 4 and 5. The parenchymal atrophy is seen to involve the lateral portions of the kidneys first (8 to 14 days), the polar areas next (28 days) and the median sagittal portion last (56 days). See figure 13.

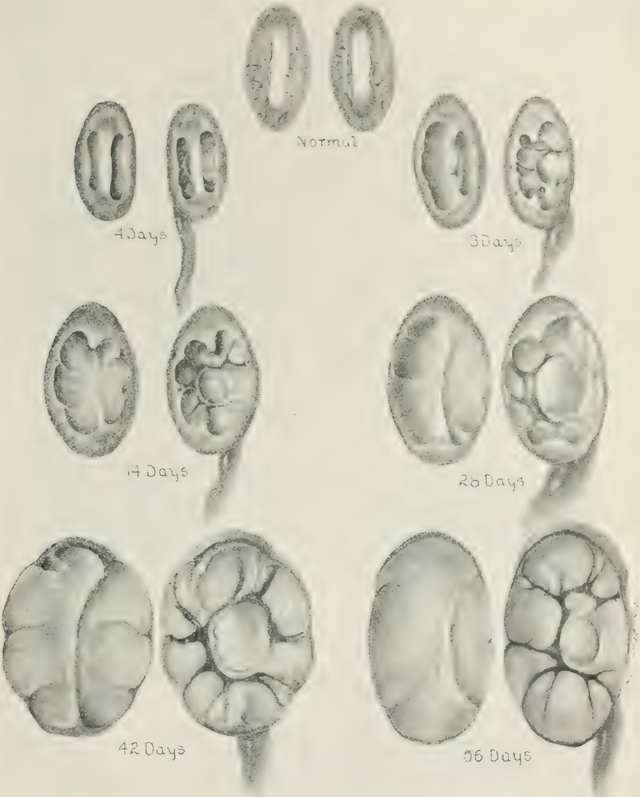

Fig. 4. Drawings of Representative Specimens of Rat Hydronephrosis Cut in Coronal Section

Compare with figures 3 and 5, also figure 13.

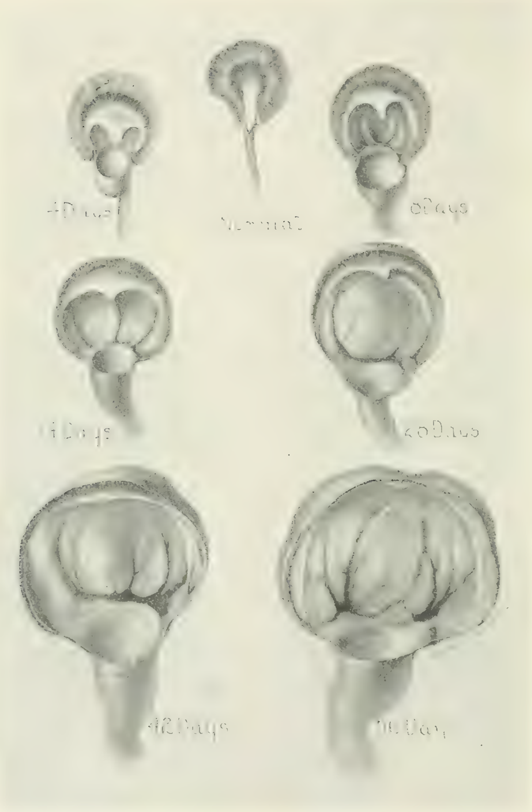

Fig. 5. Drawings of Representative Specimens of Rat Hydronephrosis of Increasing Periods of Duration, Cut in Cross Section

Compare with figures 3 and 4 and with the photograph of the specimens themselves, shown in figure 13.

strated by these measurements and is illustrated in figures 3, 4 and 5 after sagittal, coronal and cross section, respectively. (See also actual photograph of specimens in figure 13.) It will be seen that the pressure atrophy progresses in the lateral portions of the kidney first, then gradually involves both poles, and that the median sagittal portion is unusually resistant.

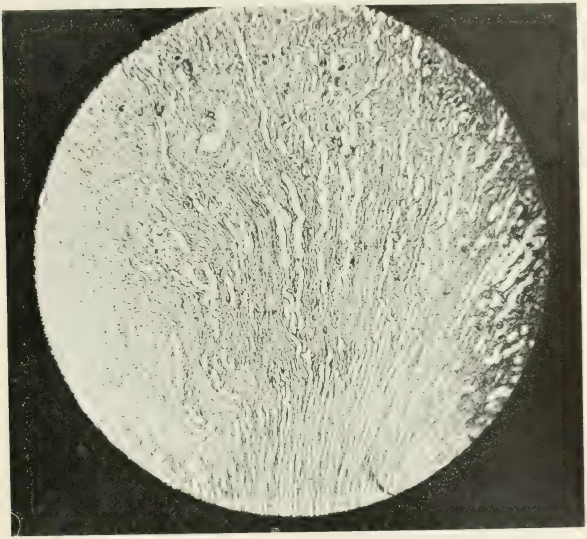

Fig. 6. Photomicrograph (Low Power) of Rat Hydronephrosis of 4 Days' Duration, Showing the Marked Tubular Dilatation Which is Most Pronounced in the Collecting Tubules and Least Noticeable in the Convoluted Tubules

The histologic changes in the rat have been described by one of us elsewhere (13). In general, there is a characteristic group distribution of the changes with areas of tubular collapse, and finally of tubular atrophy and necrosis with connective tissue sclerosis variously predominating, and there is not a uniform mass involvement. These group changes are most marked first in the lateral, then in the polar, and finally in the median sagittal portions. Collecting and straight tubules show the most marked dilatation (fig. 6), and convoluted tubules are predominatingly collapsed (figs. 7 and 8). The axes of dilated tubules in the earlier periods of obstruction are at right angles to the kidney surface (figs. 6 and 9), but after 28 days they take a course almost parallel to its surface (figs. 10 and 11). Glomeruli are surprisingly resistant and are crowded together near the

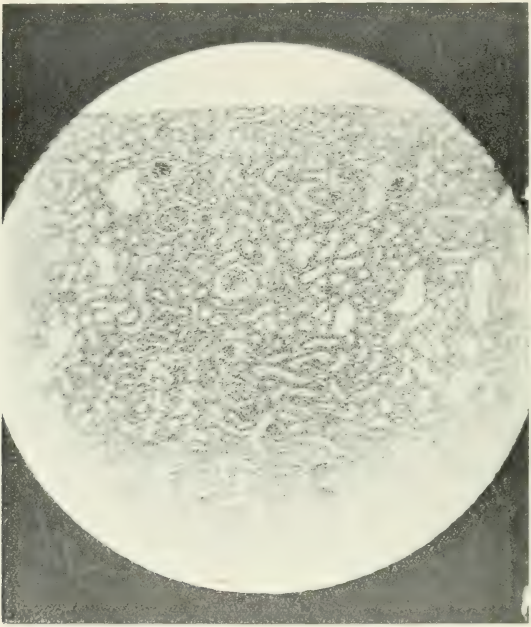

Fig. 7. Photomicrograph (Low Power) of Rat Hydronephrosis of 8 Days' Duration

Tubular dilatation is marked except for areas of convoluted tubules which are collapsed. There is dilatation of some Bowman's spaces.

surface and are often well preserved even after 95 days' obstruction and almost complete disappearance of tubules (fig. 12). One wonders how their contents can find an outlet through the dense connective tissue separating them from the pelvis (fig. 12), and yet there must be an outlet even beyond the period of one year's obstruction. The sequence of the alterations are well illustrated in the reproduced photomicrographs (figs. 6 to 12) and described in the legends.

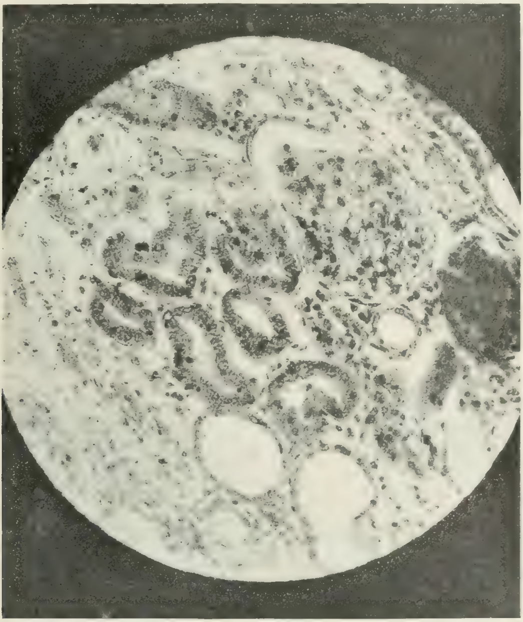

Fig. 8. Photomicrograph (High Power) of an Area of Figure 7, of an 8 Days' Rat Hydronephrosis Which Shows the Difference of Reaction of Convoluted Tubules and of Other Tubular Portions to Back Pressure
Vituaritol Stain

The convoluted tubules are collapsed or only slightly dilated.

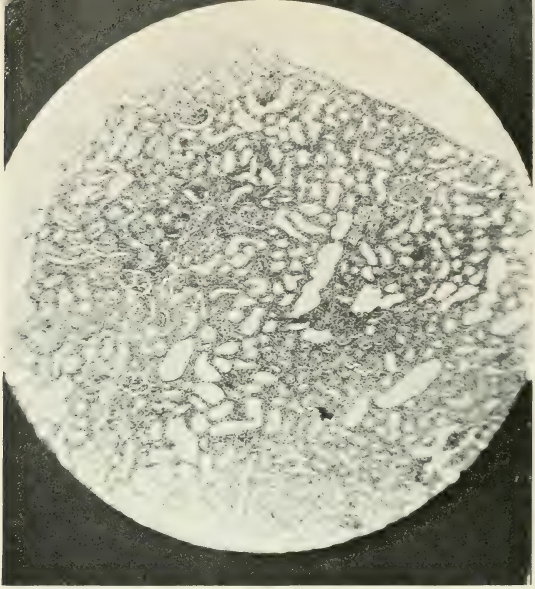

Fig. 9. Photomicrograph (Low Power) of 14 Days' Rat Hydronephrosis.

There is some dilatation with epithelial flattening of the convoluted tubules, with tendency to group-area arrangement of tubular dilatation and atrophy and of tubular preservation without much atrophy. The long axes of dilated tubules are predominatingly at right angles to the kidney surface.

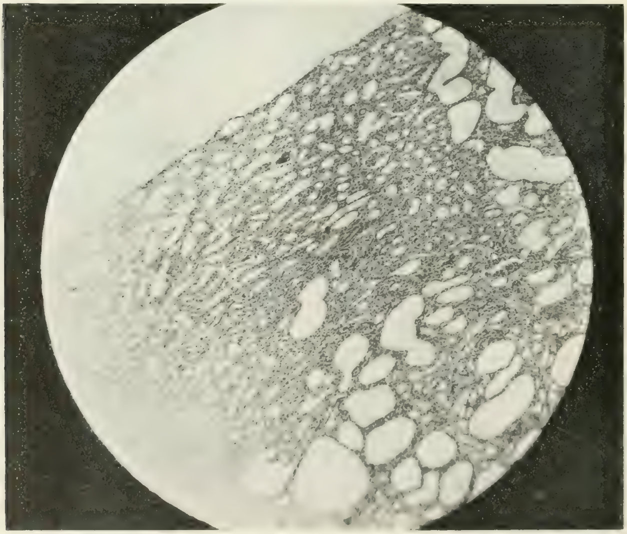

Fig. 10. Photomicrograph (Low Power) of a Rat Hydronephrosis of 28 Days' Duration, over an Area of Relative Tubular Preservation

Remarkable tubular dilatation surrounds the area. There is a distinct tendency for the axes of tubules to run parallel to the kidney surface. A few convoluted tubules show little if any dilatation.

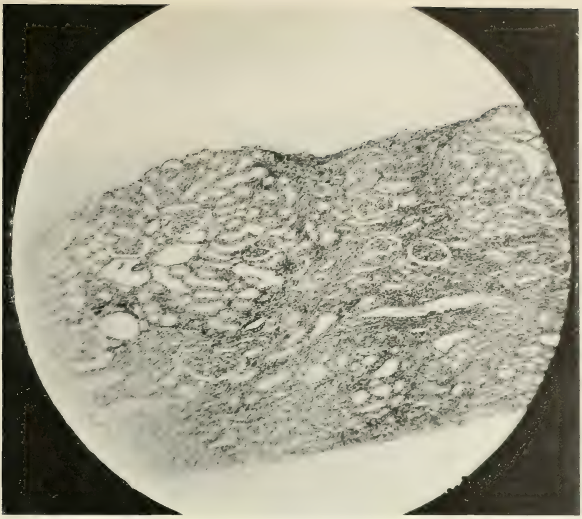

Fig. 11. Photomicrograph (Low Power) of a Rat Hydronephrosis of a 42 Days' Duration

The renal parenchyma has become a thin rim of tissue with a distinct nodulation. Marked atrophied and sclerosed areas separate a few nodules of less marked tubular destruction. Two such nodules are seen in the illustration. There is marked round cell infiltration and connective tissue substitution.

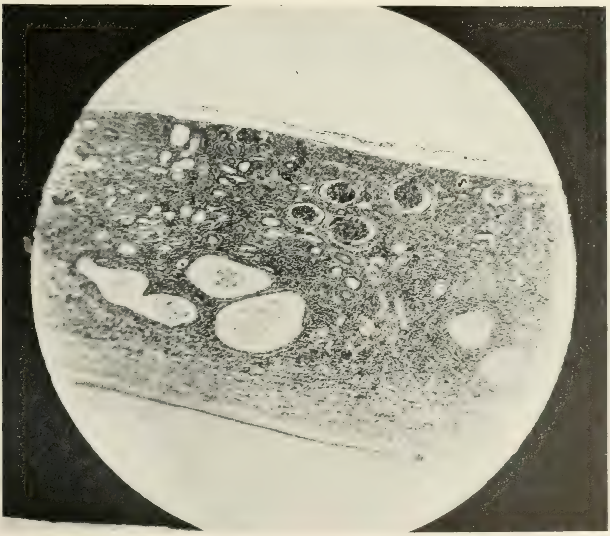

Fig. 12. Photomicrograph (Low Power) of a 56 Days' Hydronephrosis

A few groups of a small number of atrophied tubules remain. Glomeruli are fairly well preserved and are crowded together near the surface. It is difficult to see how the contents of these glomeruli gain access to the pelvis through this dense connective tissue rim.

Hydronephrotic left kidneys of rats

SERIES PROCEDURE	4 DAYS					8 DAYS			
	Rat number	Weight	Length	Width	Depth	Rat number	Weight	Length	Width
Control: Food and water freely accessible in cage throughout period	18	240	2.00	1.10	1.20				
Average		240	2.00	1.10	1.20				
Water starvation: Free access to dry food. Water given in minimal quantities in moist bread	35	245	1.80	1.08	1.10	36	210	2.65	1.5
						41	185	2.15	1.4
						45	244	2.38	1.2
						66		2.00	1.1
						67		2.10	1.2
Average		245	1.80	1.08	1.10		213	2.26	1.3
Saline diuresis: 2 per cent saline 25 cc. intraperitoneally daily. Free access to food and water									
Average									
Urea diuresis: 25 grams urea in 25 cc. water intraperitoneally daily. Free access to food and water	19	208	1.85	1.05	1.15	28	195	2.00	1.10
	21	210	1.80	1.00	1.00	29	210	2.13	1.22
Average		209	1.82	1.02	1.08		202	2.07	1.16
Diuretin diuresis: 0.05 gram diuretin in 25 cc. water daily intraperitoneally. Free access to food and water	72	240	1.85	1.00	1.00	74	250	2.30	1.20
	73	245	1.85	1.05	1.05	75	240	2.00	0.80
						76	245	2.20	1.10
						77	250	2.10	1.10
Average		242	1.85	1.02	1.02		246	2.30	1.05
Digitalis diuresis: 4.5 cc. of digitalis infusion in 25 cc. water intraperitoneally daily. Free access to food and water	49	156	1.85	0.87	0.97	52	170	2.00	1.02
	50	178	1.75	1.00	0.90	53	200	2.13	1.19
	51	170	1.80	1.06	1.00	54	170	2.00	1.02
Average		168	1.80	0.97	0.94		180	2.06	1.08
General average		210	1.84	1.02	1.04		214	2.15	1.12

mplete ligation of left ureter at bladder

14 DAYS				28 DAYS					42 DAYS					56 DAYS				
Weight	Length	Width	Depth	Rat number	Weight	Length	Width	Depth	Rat number	Weight	Length	Width	Depth	Rat number	Weight	Length	Width	Depth
240	2.40	1.30	1.20	25	240	2.60	1.60	1.90	13	246	2.50	1.40	1.30	6	243	2.20	1.50	1.80
242	2.30	1.50	1.20	26	243	2.50	1.80	1.60	15	240	2.80	1.50	1.90	88	238	2.30	1.55	0.95
243	2.50	1.40	1.30	27	252	2.70	2.00	1.70	11	320	2.35	1.55	1.15	89	237	2.65	1.63	1.60
150	2.60	1.30	1.20	8	327	2.70	1.80	1.50	14	175	2.80	1.75	1.60					
250	2.00	1.30	0.84	9	243	3.00	1.80	1.70										
225	2.36	1.36	1.15		261	2.70	1.80	1.68		245	2.61	1.55	1.49		239	2.38	1.56	1.45
243	2.10	1.00	1.25	22	230	2.10	1.70	1.30	23	240	2.50	1.70	1.40	24	233	2.40	1.50	1.30
245	2.40	1.40	1.50	68	243	2.70	1.80	1.20	90	242	2.80	1.80	1.54	70	250	3.30	2.20	2.10
250	2.30	1.45	1.45	69	256	2.70	1.70	1.60	91	239				71	250	2.40	1.80	1.40
	2.50	1.50	1.35															
246	2.33	1.33	1.39		243	2.50	1.73	1.37		240	2.65	1.75	1.47		244	2.70	1.83	1.60
200	2.20	1.30	1.10	95	180	2.75	2.00	1.53	86	261	2.90	2.10	1.80	98	183	2.87	1.87	1.50
170	2.00	1.10	1.20	96	280	2.70	1.76	1.40						87	186	3.05	1.80	1.70
151	2.05	1.12	1.10	97	287	2.50	1.71	1.34										
				84	169	2.70	1.70	1.55										
				85	270	2.50	1.60	1.24										
174	2.08	1.17	1.13		237	2.63	1.75	1.41		261	2.90	2.10	1.80		184	2.96	1.83	1.60
237	2.30	1.30	1.30	34	200	2.50	1.70	1.30	16	208	2.50	1.50	1.80	1	225	2.90	2.15	2.16
220	2.90	2.10	1.90	30	254	2.50	1.55	1.42	17	228	2.85	1.60	1.80	99	215	3.40	2.50	2.10
201	2.30	1.50	1.40															
219	2.50	1.63	1.53		227	2.50	1.63	1.36		218	2.62	1.55	1.80		220	3.15	2.32	2.13
220	2.25	1.30	1.30	31	240	2.60	1.70	1.40	12	195	2.25	2.00	1.65	10	278	2.90	1.70	1.50
270	2.38	1.50	1.30	32	250	2.20		1.30	80	245	2.40	1.60	1.30	83	280	3.10	2.50	1.80
				33	240	2.60	1.70	1.40	81	250	3.20	2.40	2.04					
				79	255	2.40	1.40	1.25	82	250	3.10	2.00	2.00					
245	2.32	1.40	1.30		246	2.45	1.60	1.34		235	2.74	2.00	1.75		279	3.00	2.10	1.65
280	2.40	1.50	1.30	58	220	2.20	1.40	1.27	60	230	3.00	1.80	2.00	64	180	3.50	2.21	2.21
290	2.55	1.75	1.55	59	228	2.35	1.25	1.15	61	222	2.50	1.70	1.30	65	217	2.95	2.02	1.85
260	2.60	1.71	1.62						62	200	3.10	2.06	1.65					
277	2.51	1.65	1.49		224	2.27	1.33	1.21		217	2.87	1.85	1.65		198	3.22	2.11	2.03
229	2.35	1.42	1.32		243	2.55	1.68	1.43		235	2.72	1.78	1.64		230	2.71	1.92	1.71

II. The Series with Oliguria and Polyuria

The results obtained with water starvation and diuresis due to salt, urea, diuretin and digitalis are so uniform that separate discussion of them is unnecessary. The detailed measurements of all specimens are given in table 2. Examination of these figures reveals no size variation between groups greater than that found between individual specimens of the control group, and these slight variations are largely nullified when proportioned to the animals' weight, as is well shown in the following summary of the 28-day groups:

		WEIGHT	LENGTH	WIDTH	DEPTH
I	Control	261	2.70	1.80	1.68
II	Water starvation	243	2.50	1.73	1.37
III	Salt	237	2.63	1.75	1.41
IV	Urea	227	2.50	1.63	1.36
V	Diuretin	246	2.45	1.60	1.34
VI	Digitalis	224	2.27	1.33	1.21

In Figure 13 is reproduced a photograph of an average series of each group, which also demonstrates the uniform nature of the enlargement throughout.

Unfortunately we cannot present experimental controls to show how the various drugs produced diuresis. Our evidence of diuresis lies in the constant observation that the cages of the animals receiving the injections were found soaking wet in a very brief period, while the cages of animals upon limited water and of the control series were constantly very dry in comparison. It was noted, furthermore, that the rats receiving urea, salt, diuretin and digitalis consumed much more of the water available in their cages than was consumed by animals of the control series. It is therefore certain that with urea, salt, diuretin and digitalis a profuse diuresis was produced.

The infusion of digitalis was selected for use because of the therapeutic preference for it as a diuretic (Janeway (14)) and its use on alternate days seems justified because of its known cumulative action (Hatcher (15), von der Heide (16)). Daily injections were found to cause, after five or six days, severe diarrhoea, necessitating a delay of a few days, and larger doses than 4.5 cc. produced a state of nervous intoxication in which the rat would react violently to sudden noises and other such stimuli, even going into strychnine-like swoons. That diuresis was produced in this group is shown by the fact that the cages of these animals were as wet as those of the animals having urea. salt and diuretin diuresis; but whether this diuresis was caused by digitalis acting in one or the other of the following four ways:

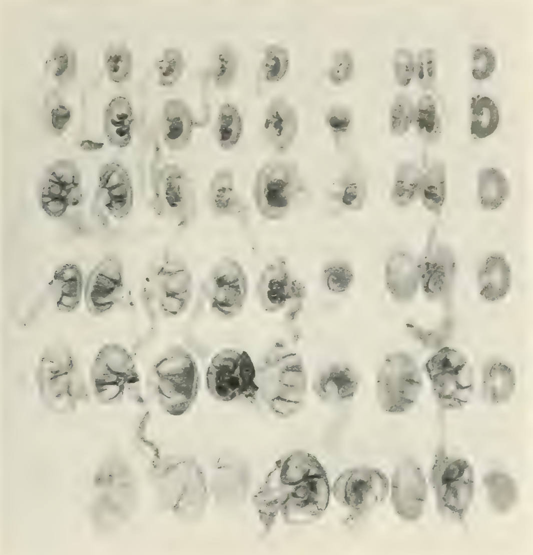

FIG. 13. PHOTOGRAPH OF REPRESENTATIVE SPECIMENS OF EACH SERIES ARRANGED IN PARALLEL PERIODS OF OBSTRUCTION

When proportioned to the weight of the animals there is little if any difference between specimens of the same period of the various groups. There is a definitely proportional amount of hydronephrotic atrophy for all, even for those showing slight differences in the amount of dilatation.

by increasing the blood pressure, by increasing the volume and altering the composition of the blood, by emptying distended venous radicles so as to lessen the resistance opposed to secretion or by directly stimulating the secretory cells of the kidney to greater activity (Lauder Brunton (17)), or was simply the result of an increased fluid intake, we are unable to say.

SUMMARY

In each of 95 young adult white rats the left ureter has been doubly ligated and completely severed just above the bladder. Following this operation the rats have been placed on a uniform diet of dry bread and grain and divided into six groups. One was allowed free access to water, another was given as little water as possible in moist bread compatible with life, and the other four groups were subjected to profuse diuresis, each getting 25 cc. of water intraperitoneally daily, and with this salt, urea, diuretin and digitalis in maximal doses, respectively. Rats in each of the six groups have been sacrificed under ether after 4, 8, 14, 28, 42 and 56 days. The measurements of the hydronephrotic kidneys of these animals show a uniform increase in size for each increasing period of ureteral obstruction. There is no variation between groups of each period greater than that occurring among the individual specimens of the control group, and this slight variation is proportional to differences in body size.

DISCUSSION

The unique nature of hydronephrosis renders a study of its mechanism unusually interesting and one inseparable from renal function. Explanation of the occasional variations in the condition itself is of interest. Why the occasional development of primary atrophy after complete ureteral obstruction in some animals and never in others and why does it never follow a partial block in any? The difference in the type of hydronephrosis that follows a complete and an incomplete obstruction is well known. What is its true explanation? The effect of blood pressure and of blood flow, of venous stasis or arterial stasis as influenced by the vascular arrangement relative to the pelvic and tubular are pertinent problems. The exact influence of these anatomical and mechanical factors upon secretory or ureteral pressure which produces the dilatation and pressure atrophy, and the secondary effect of both of these upon the filtering membrane of the glomerulus, upon the vital activity of tubular reabsorption, upon the osmotic resistance of fluid in the tubules and the rate of tubular flow must all be taken into account. The significance of the development or not of a collateral capsular circulation and the little understood but frequently observed occurrence of direct venous reabsorption from the pelvis may be circulatory factors of moment. The virulence, type and location of infection might produce variations, and the question of an occasional difference in the nervous influences affecting renal activity deserves consideration. These many and various factors emphasize its apparent complexity and little help is gained by correlation with theories of renal activity.

Any theory presupposing a purely secretory activity in the formation of the urine falls short at the very beginning by the lack of an analogy in other glands indisputably secretory.

Reasoning from gland physiology one would expect primary atrophy invariably to follow complete ureteral obstruction. Charcot and Gomboult (1), in 1878, experimentally demonstrated the difference between the kidney and other glands by the production in the guinea pig of a large hydronephrosis upon ligature of the ureter, whereas cirrhous atrophy with icterus followed ligature of the bile duct. The liver and kidney are organs quite different in structure and function and if the effect of duct obstruction in them were similar it would be surprising. But Arnozan and Voillard (2), in 1881, recorded atrophy of the parotid gland in two instances respectively of 13 and 30 days' obstruction by ligature of the duct of Stenson. The ducts themselves were found distended with normal looking saliva; many of the acini were dilated and their cells flattened in evidence of back pressure. There was epithelial desquamation, marked periglandular connective tissue sclerosis, and the obstructed glands weighed less than those on the opposite side, but nothing at all analogous to the pelvic dilatation and renal atrophy of hydronephrosis. Claisse and Dupre (3) and others have since verified these findings with respect to the parotid gland. Arnozan and Voillard also studied the effect of complete obstruction of the canal of Wirsung. The digestive action of the pancreatic secretion so hastens sclerosis that it was as far advanced in the pancreas on the third day as it was on the thirtieth in the parotid. Opie (4), while confirming these findings, observed the occurrence of small cysts in the pancreas which are interpreted by him as evidence of an intermittent type of obstruction, and both Dupre (5) and Lahousse (6) note similar pictures in the liver after bile duct obstruction. These retention cysts have a certain similarity to hydronephrosis, as pointed out by Albarron (7), in that the pressure of secretion causes a dilation of the glandular lumina, with flattening and desquamation of their preserved epithelial lining. The cyst-like accumulations in the parotid, pancreas or liver, the duct and acinal dilation, and the rapid sclerotic atrophy following these initial dilatations seem a far-fetched analogy to the progressive development of the immense fluid sacs and the gradual pressure atrophy of the renal parenchyma as seen in hydronephrosis.

Simple physical processes are likewise inadequate in explanation. No one would attempt to explain the many facts of urine excretion by filtration alone, and anyway, while mechanical filtration might explain to the satisfaction of some the formation of an hydronephrosis upon partial obstruction of the ureter, it cannot be regarded even by them as sufficient to explain the complete hydronephrotic atrophy and the huge sacs that develop after a complete ureteral block. The idea of glomerular filtration plus tubular diffusion (Ludwig) is equally unsatisfactory. While the modern theory (Cushny), that of glomerular filtration and tubular reabsorption, offers the most reasonable basis of explanation of the mechanism of hydronephrosis, there still remain many points needing explanation. Filtration may furnish the force to maintain intra-pelvic and ureteral pressure. and reabsorption in the tubules, by relieving this pressure, may thus enable a continuation of the action of this force so that pressure atrophy of the renal parenchyma with pelvic dilatation is the result, but there is no definite proof of this.

It would be profitless, however attractive, to discuss in relation to the modern theory the various theoretical aspects of the experiments with water starvation and of those with attempted salt, urea, diuretin and digitalis diuresis. This would be justified if we had knowledge as to the exact mode of action of the different drugs in producing diuresis. The uniform development of hydronephrosis, irrespective of a scant or a free flow of urine, can be interpreted so variously and in conformity to all individual predilections in theory as to give no definite proof of any, so that while the conclusion from the experimental study is quite definite, it is yet definitely limited.

CONCLUSION

Neither profuse polyuria nor marked oliguria influences in any way the usual course of development of hydronephrosis in white rats after complete ureteral obstruction.

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Reprinted from
The Journal of Infectious Diseases, Vol. 31, No. 2, August, 1922, pp. 159-197

THE PATHOGENICITY OF B. MELITENSIS AND B. ABORTUS FOR GUINEA-PIGS

STUDIES ON THE GENUS BRUCELLA NOV. GEN. IV

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In a preliminary communication [1] it was demonstrated that 5 old stock strains of B. melitensis inoculated by the intratesticular route produced in guinea-pigs anatomic lesions which could not be distinguished from those seen in this species infected with B. abortus. In fact, the pathogenicity tests furnished additional evidence to support the contention of recent workers that B. melitensis and B. abortus are closely related. It was shown that the etiologic agent of undulant fever of man persists for at least 2 months in the tissues of infected animals and exhibits a peculiar affinity for the spleen, lymphnodes, liver, and sex organs. Comparative serologic studies with the serums of the infected animals and the cultures obtained from their organs conclusively disproved any assumption that the infections might have been caused by accidentally ingested abortion bacilli, instead of by the inoculated B. melitensis. In order to verify the preliminary observations, a total of 22 strains of B. melitensis, obtained from English, Algerian, and Italian laboratories, was subsequently tested on guinea-pigs. Some of the findings necessitated a comparative study of the lesions produced by B. abortus. It has therefore been considered advisable to include in this paper data on the experimental guinea-pig disease produced by the bacteria of the Brucella group which have been collected in this laboratory during the last 6 years.

METHODS

The cultures used to inoculate the guinea-pigs were grown on glycerol peptic digest agar (P_H 6.8-7.2); only the tubes seeded with B. abortus cultures were sealed. The injections were made either into the abdominal cavity or into the parenchyma of the testicle. The latter mode of infection was carried out as follows: The anterior limbs and the left leg of the guinea-pig were held by an assistant,

Received for publication, April 26, 1922.

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[1] Proc. Soc. Exper. Biol. and Med., 1919, 16, p. 757.

and the animal was placed on its back. The operator fixed with the left hand the right or left testicle in the scrotum; occasionally gentle pressure on the abdominal wall close to the pelvic ring was necessary to bring the organ into proper position. The skin was thoroughly cleansed with alcohol and ether and then painted with iodine, or better, with a mixture of brilliant green and crystal violet in 50% alcohol; a fine needle was inserted through the scrotum into the parenchyma and, as a rule, 0.2-0.5 c c of the bacterial suspension was injected. The fingers of the left hand controlled the location of the needle and the intratesticular tension; particular care was exercised to prevent a rupture of the punctured testicle by the rapid injection of excessive amounts of the inoculum. The needle puncture was closed with iodized collodium.

The course of the infection was followed by regular weight determinations and skin tests. The preparation of the antigens, the method of application, and the interpretation of the cutaneous reactions have been stated in the previous papers.[2] When the animals were considered ready for bacteriologic examinations, they were chloroformed, necropsied and the lesions noted; portions of the tissues were preserved for sections, and cultures were prepared from the spleen, liver, kidneys, lymphnodes, bonemarrow, urine, bile, abscesses, and bone lesions. Sheep-blood-peptic digest agar plates and peptic digest agar slanted were the mediums employed. Pieces of tissue of guinea-pigs infected with B. abortus were rubbed over the entire surface of the agar slant, while sections of organs of the animals inoculated with B. melitensis were spread over blood plates. The tubes were sealed with paraffin. The colonies were identified by microscopic examination and by carefully conducted agglutination tests. Specific antiserums unabsorbed and absorbed with different strains were continuously used, and the procedures were followed, as outlined in paper II.[3] Complete agglutination tests were also carried out with the serum of the sacrificed guinea-pigs. It is evident that the existence of an infection in the guinea-pigs was considered proved only by the presence of (1) positive skin reactions; (2) distinct anatomic changes in the spleen, the liver, the lymphnodes, the testes, etc.; (3) microscopic changes in the form of focal lesions consisting of nests of actively growing epithelioidal cells and connective tissue hyperplasia; and (4) positive serum reactions, particularly agglutination reactions in dilution above 1 : 100.[4] The nature of the etiologic agent responsible for the above lesions and reactions, whether B. abortus or B. melitensis, was decided by (5) isolation of the bacteria from the tissues; (6) identification of the organisms by agglutination tests with serums specific for the various groups of the genus Brucella; (7) by absorbing the serum procured from the infected guinea-pigs with various antigens; and (8) by "passage" experiments. In the latter tests, either cultures obtained from the tissues on the first generation or the emulsions of portions of the organs were inoculated into guinea-pigs. Previous tests have demonstrated that B. abortus infections are always successfully perpetuated in this manner and that, as a rule, the anatomic lesions become more striking in the course of these "passages."

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[2] Amer. Jour. Dis. Child., 1918, 36, p. 268; 1919, 38, p. 577.

[3] Feusier and Meyer: Jour. Infect. Dis., 1920, 27, p. 185.

Early in the course of the experiments, the observations of Surface[5] on spontaneous B. abortus infections among guinea-pigs under crowded laboratory conditions were recalled and caused a feeling of uncertainty. In spite of the fact that such spontaneous epizootics, due to organisms of the Brucella group, had not been encountered among the many thousands of guinea-pigs observed during the last six years in our laboratory, the laborious and time-consuming application of all the methods mentioned was considered necessary in order to avoid even the suspicion of possible spontaneous infection. Eliminating all conceivable sources of error, the data which will be presented conclusively demonstrate that B. melitensis can provoke in a certain percentage of guinea-pigs an infection indistinguishable from abortion disease .

EXPERIMENTS WITH B. MELITENSIS

Most of the publications on undulant fever convey the impression that attempts to excite a lethal infection in the usual laboratory rodents are for the most part unsuccessful, and in order to produce a fatal disease it is necessary to introduce enormous numbers of organisms subcutaneously or intraperitoneally. It is said that the infection follows a protracted course, and weeks, even months, may pass before death occurs. A careful review shows that at least for the guinea-pig certain definite lesions have been noted by a number of observers.

Durham,[6] in 1898, by intraperitoneal injections, produced in guinea-pigs a number of infections which were chronic in character. He demonstrated B. melitensis in the urine and in the intensely engorged kidneys of these animals, which died or were killed long periods (74-94 days-7 months) after inoculation.

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[4] Repeated cutaneous injections of bacterial proteins may cause the appearance of agglutinins.

[5] Jour. Infect. Dis., 1912, 11, p. 464.

[6] Jour. Pathol. & Bacteriol., 1899, 5, p. 377.

In two instances, the spleen was much enlarged, but in the others the organ showed nothing abnormal; however, the animals were invariably emaciated. The serum of the inoculated guinea-pigs agglutinated B. melitensis in high dilutions (for example, 51st day, 1 : 200; 74th day, 1 : 1000). The same worker also injected guinea-pigs by the subdural route with cultures whose virulence had been increased by previous intracerebral passage, a method which had originally been used by Cantani to raise the virulence of B. influenzae. The course of the infection was acute, and in the animals that died the organisms were isolated from the viscera.

In 1904, Carbone,[7] who later died of undulant fever contracted in his laboratory, showed that male guinea-pigs often developed a purulent inflammation of the tunica vaginalis, following intraperitoneal injection of B. melitensis.

It is to Eyre,[8] however, that most credit is due for having studied the B. melitensis in relation to guinea-pig infections. He showed that following the first isolation from human cases, the B. melitensis could kill guinea-pigs only when given subcutaneously, intravenously, or intraperitoneally in tremendous doses. Infection was also possible by intracranial inoculation. Using a freshly isolated strain the infection was chronic, the animals living from 100 to 200 days. By repeated intracranial injections he was able to increase the virulence of the organisms, so that the pigs died within 24 to 72 hours after an intravenous injection, 3 to 12 days after an intraperitoneal inoculation, and 9 to 36 days after an intracranial inoculation.

Eyre described two types of infection in guinea-pigs, the acute and chronic, as follows:

Acute Melitensis Infection.—"Several days after a moderate dose of a highly virulent culture, or a heavy dose of a less virulent culture, the animal dies. For from two to three hours after the injection, he seems to be all right and eats well, but loss of weight sets in, which is highly characteristic for the disease. At first, the temperature drops rapidly, then rises. The animal sits huddled up, will not eat, becomes weaker and, if disturbed, goes into tonic convulsions. This stage of increased irritability goes gradually into a stage of coma, from which the animal can be aroused with difficulty, and in which he remains about 24 hours with a subnormal temperature. Sometimes he has a convulsion before death, but usually it occurs without warning. The postmortem findings are those of acute septicemia. The organisms can be recovered from all the tissues and organs. In male animals, following intraperitoneal infections, there develops a bilateral purulent process in the tunica vaginalis with beginning hypertrophy of the testes. This pus contains numerous organisms."

Chronic Melitensis Infection.—"With small doses of virulent, or fair doses of moderately virulent organisms, the animal gets a chronic infection. Aside from a progressive loss of weight and a severe anemia, there are no characteristic symptoms. Even the temperature is not much changed. The incubation period of the chronic variety of the disease is from two to three days. Then comes a time, three to six days, when the animal is perceptibly sick. He will not eat, stays in a corner of his cage, loses weight and is very weak. He gradually improves, eats well, even ravenously, and although losing no more weight, he never regains his original weight. Following weeks or months, during which, aside from the loss of weight, the animal seems well, he suddenly dies. Postmortem cultures from the liver are sterile; from the spleen and bonemarrow there is sometimes a scanty growth, although these are also often sterile. From the kidneys and the urine a good growth of B. melitensis is obtained."

[7] Arch. per le sc. med., 1904, 28, p. 273.

[8] Report Mediterranean Fever Commission, 1905, Part 2, p. 67.

Nicolle and Conseil,[9] in 1909, reported on 2 guinea-pigs, which had naturally acquired a B. melitensis infection in a stable, where infected maltese goats had been kept. The serum of both animals agglutinated B. melitensis in a dilution of 1 : 300 and the specific organism was isolated from the spleen and liver of one guinea-pig. The blood, urine, and bile were sterile. In a subsequent paper[10] these two investigators recorded some inoculation experiments on guinea-pigs with a strain recently isolated from the milk of an infected goat and they stated that "Contrairement a l'opinion classique, nos expériences ont donné constatement un resultat positif; le cobaye est donc un animal parfaitement sensible au M. melitensis." One guinea-pig, which had been injected with the growth of one ¼ agar slant and sacrificed on the 138th day, exhibited a slight hypertrophy and a granular appearance of the spleen. The same findings were made on a similarly treated guinea-pig, which was killed on the 42nd day. Feeding of cultures to 2 guinea-pigs for 10 consecutive days produced the same lesions. Spleen cultures were always positive, while B. melitensis was also isolated from the urine of 2 animals. The serum agglutinated the inoculated organism in dilutions of from 1 : 250 to 1 : 1000.

Sangiorgi (1913)[11] inoculated 3 guinea-pigs intraperitoneally with the blood of an undulant fever patient. The blood of 2 of the animals, when killed on the 30th day, agglutinated B. melitensis in a dilution of 1 : 100, and the spleens were greatly enlarged and soft ("la milza era molto in grandita, friable"). In a recent study by Tallo,[12] it is shown that B. melitensis may persist for at least 35 days in the gallbladder and bile of guinea-pigs, which have been injected intravenously. These observations confirm those of Eyre and others and show that B. melitensis may leave the body by way of the alimentary canal, in the intestinal mucus, and in the feces.

It is evident, from this review, that guinea-pigs are susceptible to B. melitensis and that a chronic disease with an enlargement of the spleen and a persistence of the inoculated bacteria in the tissues and secretions has been repeatedly noted. The significance of these facts has, however, not been appreciated until a study of B. melitensis by guinea-pig inoculations. Preliminary experiments,[1] conducted by the writers, resulted in negative postmortem and cultural findings until a special mode of injection, namely, the intratesticular route, was chosen. Some observations, which will be detailed in a subsequent chapter, had indicated that B. abortus possessed a striking affinity for the sex organs. As the two bacteria, B. abortus and B. melitensis, were morphologically, biochemically and serologically closely related, it was suspected that the sexotropic pathogenicity of B. abortus might also be characteristic of the organism of undulant fever and the possibility, therefore, of producing specific lesions by injections of the virus into the testes suggested itself. The majority of the inoculations reported in the following were made intratesticularly. Successful infections have, however, also been noted following intraperitoneal injections. The data dealing with the observations on 203 guinea-pigs are also presented in the following.

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[9] Compt. rend. Soc. de biol., 1909, 66, p. 503.

[10] Ibid., 1909, 67, p. 267.

[11] Pathologica, 1913, 5, p. 554.

[12] Ibid., 1919, 11, p. 401.

EXPERIMENTS WITH B. MELITENSIS STRAINS 1, 2, 3, 4, 5, 7, 8, 9, 10, 23, 24 and 25[13]

One tenth slant of a glycerol peptic digest agar culture was inoculated into the right testicle of guinea-pigs; as a rule, a series of 4 to 6 animals were infected. A total of 60 guinea-pigs was used; they all survived the infection and were sacrificed at different times after the inoculation, the period varying from 6 weeks to 6 months. Repeated skin tests gave negative reactions. At necropsy, the tissues were free from lesions, and the serum failed to agglutinate the strain of B. melitensis used for the inoculations in dilutions greater than 1 : 40.

EXPERIMENTS WITH A POLYHOMOGENOUS MIXTURE OF B. MELITENSIS STRAINS 2, 3, 4, 5, AND 11

The results on the first series of 8 guinea-pigs have already been reported in a previous communication.[1] The cultures obtained from the tissues of 2 guinea-pigs (614 and 615) which exhibited typical necropsy findings were used for further pathogenicity tests. The results are tabulated below.

Melitensis Culture from Urine and Testis of Guinea-Pig 614 Inoculated Intratesticularly

Guinea-Pig 654.—Sacrificed on 119th day; skin tests always positive; necropsy revealed small spleen, but typical lesions in lymphnodes and liver.

Guinea-Pig 655.—Died on 45th day with typical lesions.

Spleen emulsion inoculated with negative results intratesticularly into four (709, 710, 711, 712) and intraperitoneally into two guinea-pigs (713, 714).

Spleen culture, 2d generation, was identified in Monkey 15 as a B. melitensis. One guinea-pig (733), of 8 inoculated, revealed a typical lesion at necropsy.

The histories of guinea-pigs 655 and 733 are presented in detail.

Guinea-Pig 655.—Weighing 540 gm., was inoculated into the right testicle with 1/20 slant of a young culture (2d generation) of B. melitensis isolated from the testis and urine of guinea-pig 614. On the 44th day after the inoculation (weight 537 gm.) the animal was subjected to a skin test with "aborto" and "melitensis protein." The following day, the guinea-pig exhibited marked skin reactions, coryza, and dyspnea; it apparently died from anaphylaxis 21 hours after the cutaneous injection of the bacterial proteins. Necropsy demonstrated an enlarged, nodular, and typical spleen (weight 1.9 gm.), large and firm lymphnodes, numerous foci in the liver and bilateral atrophy of the testes. The microscopic findings were characteristic. B. melitensis was isolated from the spleen and the left precrural lymphnodes; the urine, bile, bonemarrow, liver, and testes were sterile. The serum gave the following agglutination reactions:

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[13] Histories of Strains: 1: marked R. A. M. C.; 2: U. S. Navy; 3: stock 22; 4: Dr. Arneth; 5: stock; all obtained from the Hygienic Laboratory, U. S. Public Health Service 1918; 7: marked R. Mum. 5; 8: marked R. Monaco; 9: R B_2 paramelitensis, obtained from Dr. E. Sergent, Institut Pasteur d'Algerie, 1919; 10: obtained from the Institute for Infectious Diseases, Bern, 1914; 23: paramelitensis R. A. M. C., obtained from the Lister Institute; 24: marked Celli obtained from Dr. A. Ascoli, Modena, Italy; 25: marked "Douglas" obtained from Dr. J. W. H. Eyre, Guy's Hospital, London, 1919.

B. melitensis 1, 2, 3, 4, 5, 8, 11 — 1:400 = 1:1000 depending on the antigen; after absorption with B. abortus 80, 1:200.

B. melitensis 7 and 9 — < 1:40.

B. abortus 80 and 320 — 1:100; after absorption with B. abortus, < 1:40.

Guinea-Pig 733.—Weighing 450 gm., was inoculated intratesticularly with 1/10 slant of a culture (1st generation) obtained from the spleen of guinea-pig 655. The skin reactions were positive. On the 155th day, the animal weighed 221 gm. and was chloroformed. The necropsy findings were typical, the spleen weighed 1.4 gm., and the liver was studded with small, grayish foci. B. melitensis was isolated from the spleen and bonemarrow and the serum agglutinated B. melitensis 11, 1:1000, B. abortus 80, 1:800 and B. melitensis 7 and 9, < 1:20.

Two guinea-pigs (706 and 707) inoculated with the culture obtained from the urine of guinea-pig 615 survived and presented negative necropsy findings. A third guinea-pig (708) died on the 30th day after the inoculation with typical lesions and positive cultural findings.

A second series of 12 guinea-pigs gave the following result: Four of 6 guinea-pigs inoculated intraperitoneally with 2,000 million bacilli of a mixture of B. melitensis strains 2, 3, 4, 5 and 11 died on the 2nd and 3rd days, respectively, after the inoculation. In every case a fibrinopurulent peritonitis was present, and the injected bacilli were isolated from every organ. The remaining 2 guinea-pigs proved negative at necropsy on the 120th day. Six guinea-pigs injected intratesticularly with the same inoculum survived and presented negative findings when sacrificed 112 days after the inoculation.

A third series of 4 guinea-pigs inoculated intraperitoneally with 1/50 slant of the mixture mentioned of strains furnished 2 guinea-pigs (807 and 809) with moderately severe chronic lesions. Two guinea-pigs died on the 16th and 26th day, respectively; B. melitensis was found in the spleen, liver, bonemarrow, bile, kidneys, and urine, but not in the heart blood. The histories of guinea-pigs 807 and 809 are detailed.

Guinea-Pig 807.—Weighing 330 gm., was inoculated intraperitoneally with 1/50 slant of a mixture of B. melitensis 2, 3, 4, 5 and 11. It was chloroformed 87 days after the inoculation when it weighed 417 gm. The spleen weighed 3.5 gm., it was nodular and soft; the edges were rounded. Numerous lymphnodes, in particular the iliac, precrural and postmanubrial nodes, were slightly enlarged and firm. A few B. melitensis were isolated from the splenic pulp; the bonemarrow, liver, bile, urine, and kidneys were sterile. The serum agglutinated the various strains as follows: B. melitensis 11, 1:100-200; B. melitensis 7, 1:80; No. 9, 1:20; and B. abortus 80, 1:200; after absorption with B. abortus 80, the serum agglutinated B. melitensis 1:60. The strain of

B. melitensis isolated from the spleen was agglutinated in a dilution of 1:200 by a group II serum after the serum had been absorbed with B. abortus No. 80 (serum titer 1:1000, after absorption 1:200 for B. melitensis group II and B. abortus 80, <1:40). An emulsion of the spleen was inoculated intraperitoneally or intratesticularly into guinea-pigs 877, 878 and 879.

Guinea-Pig 809.—Weighing 685 gm., was inoculated intraperitoneally as guinea-pig 807. It gave a strongly positive skin reaction with B. melitensis and B. abortus protein on the 76th day after the injection. The guinea-pig was sacrificed on the 97th day when it weighed 676 gm. The postmortem findings were typical; the spleen weighed 2.2 gm.; it was nodular and pulpy. The lymphnodes were enlarged and hard. B. melitensis was isolated from the spleen, while the urine, bile, bonemarrow, etc., were sterile. The serum agglutinated the various B. melitensis strains as follows: B. melitensis 11, 1:200; B. melitensis 7 and 9, 1:100; and B. abortus 80, 1:200; when absorbed with B. abortus No. 80, the serum agglutinated only B. melitensis 11 in a dilution of 1:100.

In a fourth series the infected spleen of guinea-pig 807 was injected intraperitoneally into guinea-pigs 877 and 878, while guinea-pig 879 was treated intratesticularly. The three animals presented typical lesions when sacrificed on the 92nd day. The abstracted histories are briefly as follows:

Guinea-Pig 877.—Weighing 575 gm., was injected intraperitoneally with 2 cc of an emulsion 1:10 of the spleen of guinea-pig 807. When sacrificed on the 92nd day, the guinea-pig weighed 526 gm. and showed a nodular, enlarged spleen (weight 1.5 gm.), numerous foci in the liver, enlarged and firm lymphnodes. B. melitensis was isolated from the spleen, kidneys and liver. The serum agglutinated B. melitensis 1, 1:400 and B. abortus 1:400; after absorption, B. melitensis 27 was clumped in a dilution of 1:100, B. abortus <1:80.

Guinea-Pig 878.—Weighing 526 gm., was inoculated as 877. The weight at necropsy was 566 gm.; the spleen was enlarged and nodular (weight 2.1 gm.); the uterus contained 3 embryos. B. melitensis was isolated from the left kidney, spleen and one placenta. The serum agglutinated strains 26 and 615 in dilution >1:200 after absorption with B. abortus 80. B. melitensis was agglutinated 1:160.

Guinea-Pig 879.—Weighing 593 gm., was inoculated intratesticularly as was guinea-pig 877. At necropsy (weight 563 gm.) the nodular spleen weighed 1.2 gm., and the left seminal vesicle was distended by stringy pus. B. melitensis was isolated from the spleen (6 colonies), urine and left kidney. The serum reactions were as follows: B. melitensis 21, 1:800; No. 11, 1:400; B. abortus, 1:100; No. 7 and 9, <1:10; after absorption with B. abortus, B. melitensis 11 was clumped 1:640 + + +.

EXPERIMENTS WITH B. MELITENSIS STRAIN 6

The strain used in these experiments was obtained in 1919 from Dr. E. Sergent, Institut Pasteur d'Algerie, and was marked "Race Souk-Alvias." Six guinea-pigs were inoculated with 1/10 slant of B. melitensis 6. One guinea-pig (639) died on the 10th day and one (638) was sacrificed on the 15th day. Both animals showed typical lesions, while the remaining 4 guinea-pigs, when sacrificed on the

140-160th day after the inoculation, exhibited normal necropsy findings. The necropsy findings of guinea-pigs 638 and 639 are detailed.

Guinea-Pig 638.—Weighing 675 gm., was inoculated into the right testicle with 1/20 slant of a young culture of B. melitensis 6. It was chloroformed 15 days after the injection, when it weighed 714 gm. Necropsy revealed a generalized enlargement of the lymphnodes and atrophy of the testis; the spleen weighed 0.5 gm., was light brownish and soft. B. melitensis was isolated from only the spleen and the right testis. The serum agglutinated B. melitensis 5, 1 : 100 + + +; while it failed to react with B. abortus 320.

Guinea-Pig 639.—Weighing 505 gm., was inoculated intratesticularly as was guinea-pig 638. The animal died on the 10th day (weight 460 gm.). The spleen weighed 1.4 gm., was nodular and showed numerous yellowish necrotic foci; similar focal necroses were seen in the liver and the iliac lymphnodes; the right testis was partially necrotized and deeply injected. Cultures demonstrated the fact that all the organs, bile and urine were teeming with B. melitensis.

EXPERIMENTS WITH B. MELITENSIS STRAIN 11

The strain was obtained in 1918 as Micrococcus melitensis 33 from the American Museum of Natural History.

Two series of (717-726 and 753-758) 5 and 6 guinea-pigs each were inoculated intraperitoneally and intratesticularly with 1/3-1/10 slant of a young agar culture. In the first series, only 2 animals, guinea-pig 720 (intratesticularly) and 724 (intraperitoneally), gave positive cultures at necropsy. In the second series, 5 of the 6 animals injected intraperitoneally died in from 4-12 days after the inoculation; they exhibited the usual lesions, the surviving animal was negative. Two guinea-pigs (753 and 755), infected by the intratesticular route, presented typical necropsy findings; one animal (754) had succumbed to an intercurrent infection; one (756) died 7 days after the injection, and 2 (757 and 758) gave negative findings. The histories of the infected guinea-pigs are detailed.

Guinea-Pig 720.—Weighing 615 gm., received intratesticularly 1/3 slant of B. melitensis No. 11. Skin tests were repeatedly positive. At necropsy examination (weight 870 gm.) 127 days after the inoculation, the spleen was small, weighing 0.9 gm., the right testis was hard and atrophic, numerous lymphnodes were slightly enlarged and firm; B. melitensis was isolated from the pus and spleen. The serum agglutinated B. melitensis 1 : 200 after absorption with B. abortus.

Guinea-Pig 724.—Weighing 462 gm., was inoculated as was 720. Two cutaneous tests were positive. Sacrificed on the 127th day (weight 722). The omentum was adherent to the liver and spleen, the spleen was small and weighed 0.9 gm. The precrural, postmanubrial and iliac lymphnodes were firm and enlarged; the testes were atrophic; B. melitensis was isolated from the precrural lymphnodes. The serum agglutinated B. melitensis 11 after absorption with B. abortus 1 : 80.

Guinea-Pig 753.—Weighing 607 gm., was injected into the right testicle with 1/10 slant of a young culture of B. melitensis 11. When chloroformed on the 112th day after the inoculation, it weighed 608 gm. and showed the following lesions: Spleen slightly enlarged, weight 1.4 gm., nodular; the precrural and iliac lymphnodes were hard and slightly enlarged; both testes were atrophic. B. melitensis was isolated from the right testis. The serum agglutinated B. melitensis 1:400 and B. abortus 1:100.

Guinea-Pig 755.—Weighing 613 gm., was inoculated in the same manner as guinea-pig 753. When sacrificed on the 112th day, it weighed 495 gm. and exhibited typical lesions. The spleen weighed 2.1 gm. and was distinctly nodular The serum agglutinated several strains of B. melitensis as follows: B. melitensis 11, 1:600; B. melitensis 7 and 9, 1:20-1:40; B. abortus 80, 1:200. B. melitensis group II was isolated from the spleen.

EXPERIMENTS WITH B. MELITENSIS STRAIN NO. 18

The strain used in these experiments was obtained from the Lister Institute; it was marked M. melitensis (Arkwright) and had been isolated in Malta in 1915 or 1916 from the blood of a patient. Two series of 7 guinea-pigs were inoculated intratesticularly. Four animals died in from 2-15 days after the inoculation and revealed at necropsy the usual lesions of a septicemia. Three animals survived and 2 (766 and 812) presented typical anatomic and cultural findings.

Guinea-Pig 766.—Weighing 460 gm., received intratesticularly 1/10 slant of B. melitensis 18. Skin tests were positive on the 82nd day. Necropsy examination on the 119th day revealed an emaciated body (weight 382 gm.), an enlarged, nodular spleen, weighing 1.6 gm., firm and enlarged lymph nodes, atrophic right testis and purulent seminal vesiculitis. B. melitensis was isolated from the pus and the spleen. The serum was lost.

Guinea-Pig 812.—Weighing 510 gm., was injected as guinea-pig 766. At necropsy (98th day, weight 490 gm.) the spleen weighed 1.8 gm., and was distinctly nodular. B. melitensis was isolated only from the spleen. The serum agglutinated B. melitensis 18, 1:200; B. abortus 80, 1:1000; B. melitensis 7 and 9, 1:60, after absorption with B. abortus 80. B. melitensis was agglutinated 1:100 and B. abortus 80, $< 1:40$.

EXPERIMENTS WITH B. MELITENSIS 19

The strain was received from the Lister Institute; the tube was marked M. melitensis Eyre—milk, malta fever.

Two series of 4 guinea-pigs each were inoculated intratesticularly. Two animals died in from 5-15 days after the inoculation. Six guinea-pigs survived and 5 presented at necropsy normal organs with negative cultural findings, while one (770) showed these lesions:

Guinea-Pig 770.—Weighing 483 gm., was inoculated with 1/10 slant of B. melitensis 19. Necropsy on the 110th day revealed numerous small abscesses in the right testis and epididymis and pus in the right seminal vesicle; the spleen was small (0.65 gm.); the lymphnodes were firm and slightly enlarged. B. melitensis was isolated only from the pus of the abscesses. The serum agglutinated B. melitensis 19 in a dilution 1:200.

EXPERIMENTS WITH B. MELITENSIS 20

The strain used in these experiments was obtained from the Lister Institute; the tube was marked M. melitensis Austria I, and it was stated that the culture was originally obtained from the Royal Army Medical College, Millbank, London. This strain belongs to our group 1 of the Brucella group and cannot be separated from the B. abortus group. In paper III the melitensis character of the organism was proved by a pathogenicity test on one monkey.

Two series of 4 guinea-pigs each were inoculated intratesticularly. One animal died on the 22nd day after the inoculation, the spleen appeared normal, but from it B. melitensis was isolated. Seven guinea-pigs were sacrificed in from 72-132 days after the infection; necropsy revealed no pathological changes.

EXPERIMENTS WITH B. MELITENSIS 21

The strain was obtained from the Lister Institute and was marked M. melitensis (Basset-Smith). According to a personal communication from Surgeon Rear-Admiral P. M. Basset-Smith, Royal Naval College, Greenwich S. E., this strain was isolated by him in 1904 from a blood culture of an undulant fever patient in his ward and sent to the Lister Institute in 1905. B. melitensis 21 belongs serologically to group 2 or the main group of the genus Brucella. The pathogenicity for guinea-pigs was tested on 3 series of animals. The first series of 4 guinea-pigs gave entirely negative results. The histories of the guinea-pigs used in the second series are presented:

Guinea-Pig 819.—Weighing 543 gm., was inoculated intratesticularly (right testis) with 1/10 slant of a young culture of B. melitensis 21. The animal was sacrificed on the 103rd day, when it weighed 445 gm. The anatomic lesions were typical; the spleen weighed 1.82 gm. (1.2 x 4.0 cm.), showed numerous grayish nodules and a deep brownish pulp; the lymphnodes were all enlarged and hard. In the kidneys a few grayish nodules were visible. The right and left testes were atrophic, the right was fibrous. B. melitensis was isolated from the spleen and kidneys. The serum of the guinea-pig agglutinated various strains of the Brucella group as follows: B. melitensis 21, 1:1000-2000; B. melitensis 7 and 9, 1:40-1:80; B. abortus 80, 1:800; absorbed with B. abortus 80, agglutinated B. melitensis 1:400 + + +.

Guinea-Pig 820.—Weighing 395 gm., was inoculated as guinea-pig 819; it was found dead 10 days after the inoculation (weight 254 gm.). The right testis was partially necrotic, covered with fibrin, the spleen was deep brownish, soft, slightly enlarged (0.9 gm.), and the bile was blood tinged. B. melitensis was isolated from the spleen, liver, urine and from the bile; innumerable colonies developed on the blood plates. No serum could be procured.

Guinea-Pig 821.—Weighing 383 gm. and treated as 819, died on the 8th day after the inoculation. The lesions and cultural findings were identical to those recorded for guinea-pig 820.

TABLE 1

Experiments with B. Melitensis 21, Series 3

Guinea-Pig Number	Injected With	Weight		Sacrificed	Anatomic Lesions	Bacteriologic Results	Serologic Findings Serum Agglutinates
		Initial, Gm.	At Necropsy, Gm.				
883 ♂	1/25 slant	630	529	70th day	Spleen 2.1 gm., nodular and typical; lymphnodes enlarged and hard; foci in liver and lungs; testis atrophic, left adhesions; microscopically typical	B. melitensis isolated from left femoral bonemarrow, spleen liver and precrural lymphnode	B. melitensis 21, 1:2000; B. abortus 1:80 after absorptions with No. 80 agglutinates B. melitensis 21, 1:640, B. abortus 80, <1:20
884 ♂	1/25 slant	598	506	70th day	Spleen 1.1 gm., both testis atrophic, left fibrous; lymphnodes slightly enlarged; 8-10th right and left sternochondral junction of ribs enlarged, nodular, containing a yellowish pus-like substance	B. melitensis isolated from two rib lesions, spleen; liver and bonemarrow sterile	B. melitensis 1:1000; B. abortus 1:200, absorbed with B. abortus agglutinates B. melitensis 1:640
885 ♂	1/25 slant	637	515	70th day	Lesions as above mainly in right ribs; spleen 0.9 gm.	B. melitensis isolated from ribs only	B. melitensis 1:1000; B. abortus 80, 1:160
886 ♂	1/25 slant	738	390 D.	41st day	General lymphadenopathy; abscess in right testis and purulent seminal vesiculitis, atrophy left testis; spleen 0.9 gm. Cloudy swelling in parenchymatous organs	B. melitensis isolated from testis and spleen	Not tested
887 ♂	1/25 slant	783	625	70th day	Spleen 1.4 gm.; 12th right rib enlarged periosteochondritis on left lower femur and upper end of tibia involving left knee. Lymphnodes not enlarged	B. melitensis isolated from spleen and rib lesion	B. melitensis 21, 1:400; B. abortus 1:400, after absorption, B. melitensis 21 1:200; B. abortus 80, <1:20
888 ♂	1/25 slant	585	595	70th day	Spleen 0.75 gm.; both testis atrophic and seminal vesicle fibrous; right and left sternochondral rib insertion enlarged as 884	B. melitensis isolated from spleen and rib lesions	B. melitensis 21, 1:2000; B. abortus 80 after absorption; B. melitensis, >1:1280; B. abortus <1:20

The exceedingly interesting and important results collected from series 3 are presented in table 1.

EXPERIMENT WITH B. MELITENSIS 22

The strain is known as B. paramelitensis (Basset-Smith) originally sent to Fleet Surgeon R. W. Basset-Smith by Dr. C. Nicolle of the Institut Pasteur of Tunis, obtained from the Lister Institute. Seven guinea-pigs were tested with this strain. Four animals died in from 10-21 days showing the usual lesions of a septicemia. Only one (822) of the 3 surviving guinea-pigs presented typical lesions at necropsy, 79-120 days after the inoculation.

Guinea-Pig 822.—Weighing 685 gm., was inoculated intratesticularly with 1/10 slant of B. melitensis 22. The same animal gave a strongly positive skin reaction and was therefore sacrificed on the 79th day after the injection (weight 538 gm.). The spleen was enlarged and weighed 1.4 gm. (2.5 x 1.5 cm.); the lymphnodes were small but hard; the left seminal vesicle contained creamy pus, and the liver exhibited a few foci. B. melitensis was isolated from the urine, spleen, seminal vesicle and both kidneys. The serum gave the following serum reactions: B. melitensis 22, 1:800 + + +; B. melitensis 11, 1:100 and B. abortus 80, 1:800 + + +.

EXPERIMENTS WITH B. MELITENSIS 26

The strain was obtained from Dr. J. W. H. Eyre, Guy's Hospital, London; it had been isolated in 1918 (?) from the spleen of a case of undulant fever. The results obtained on 9 guinea-pigs are detailed:

SERIES 1.—*Guinea-Pig 834.*—Weighing 480 gm., was inoculated intratesticularly with 1/10 slant of B. melitensis 26. The animal was sacrificed on the 108th day after the inoculation (weight 355 gm.) and revealed typical lesions. The spleen weighed 1.4 gm. (0.8 x 2.2 cm.), and the lymphnodes were slightly enlarged. The right testis was atrophic and fibrous. B. melitensis was isolated from the spleen. The serum agglutinated the various available strains as follows: B. melitensis 26, 1:1000 + + +; B. abortus 80 and B. melitensis 7 and 9 in a dilution of 1:40.

Guinea-Pig 835.—Weighing 440 gm., was inoculated in the same manner as guinea-pig 834. The animal died on the 25th day after the inoculation (weight 320 gm.); the right testis contained an abscess; the regional lymphnodes were enlarged and firm; the spleen was small (0.5 gm.). B. melitensis was isolated from the pus of the testicular abscess, the bonemarrow, the bile and the spleen. No serum was obtained for agglutination tests.

Guinea-Pig 836.—Weighing 360 gm., was inoculated intratesticularly with 1/10 slant of B. melitensis 26. The animal died on the 28th day; it was emaciated and weighed 225 gm. Necropsy revealed a spleen weighing 1.3 gm., which was soft and light brown. The lymphnodes appeared normal, while the testes were atrophic; the right testis in particular was rather fibrous. The right epididymis contained creamy pus. The gallbladder was distended and contained blood tinged bile. B. melitensis was isolated from the spleen, epididymis, bile and left femoral bonemarrow. No serum could be procured for agglutination tests.

TABLE 2

Experiments with B. Melitensis 26, Series 2

Guinea-Pig Number	Injected With	Weight		Sacrificed	Anatomic Lesions	Bacteriologic Results	Serologic Findings Serum Agglutinates
		Initial, Gm.	At Necropsy, Gm.				
889 ♂	1/20 slant	526	405	70th day	Spleen 2.5 gm.; (3.3 × 2.2) typical lymphadenitis; atrophic testis; foci in liver	B. melitensis isolated from spleen and bonemarrow	B. melitensis 26, 1:400; B. abortus 80, 1:100, absorbed with B. abortus 80, B. melitensis 1:200; B. abortus <1:40
890 ♂	1/20 slant	873	833	Died 13th day	Spleen pulpy, 1.4 gm.; necrosis of right testis; cloudy swelling of liver and kidneys	B. melitensis isolated from spleen, liver, kidneys, bile, bonemarrow	Not tested
891 ♂	1/20 slant	463	541	78th day	Spleen 1.6 gm.; one nodule; slight lymphadenopathy; right testis atrophic	B. melitensis only in spleen......	B. melitensis 1:200; B. abortus 80, 1:100; absorbed <1:40
892 ♂	1/20 slant	519	448	61st day	Spleen small, 0.8 gm.; slight enlargement of sternochondral junction of right 5th, 6th, 7th and 8th rib; other organs normal. Right testis small focus of necrosis	B. melitensis only from right testis	B. melitensis, 1:60; B. abortus 80, 0
893 ♂	1/20 slant	618	603	Died 6th day	Hemorrhagic orchitis; intoxication	B. melitensis isolated from spleen, liver and bile; no other organ tested	Not tested
894 ♂	1/20 slant	513	475	70th day	Spleen 2.4 gm., typical; lymphadenopathy; atrophic right testis, abscess in left epididymis; foci in liver	B. melitensis isolated from spleen and seminal vesicle	B. melitensis 26, 1:1000; B. abortus 80 absorbed; B. melitensis, 1:400(?-600); B. abortus 80, 1:40

EXPERIMENTS WITH B. MELITENSIS 27

The strain used in these experiments was obtained from Dr. J. W. H. Eyre, Guy's Hospital, London; the culture had been isolated in 1917 or 1918 from goat's milk. The results obtained on 8 guinea-pigs are detailed:

SERIES 1.—*Guinea-Pig 837.*—Weighing 490 gm., was inoculated intratesticularly with 1/10 slant of B. melitensis 27. The animal died on the 57th day when it weighed 317 gm. The lesions were typical. B. melitensis was isolated from the spleen (∞ colonies), right testis and seminal vesicle and the bile.

Guinea-Pig 838.—Weighing 428 gms., was inoculated in a similar manner as guinea-pig 837. It was found dead on the 57th day, when it weighed 249 gm. The lesions resembled those recorded for guinea-pig 837. B. melitensis was isolated from the spleen, right testis and the bile.

Guinea-Pig 840.—Weighing 728 gm., was inoculated intratesticularly with 1/10 slant of B. melitensis 27. On the 76th day after the inoculation, the animal gave strongly positive skin reactions with B. abortus and B. melitensis protein. The guinea-pig was sacrificed on the 99th day, when it weighed 694 gm. A typical nodular, enlarged spleen, weighing 2.3 gm. (3.2 x 2.5 cm.) was found. The liver was studded with numerous grayish areas, and all the lymphnodes were slightly enlarged and firm. The right testis and seminal vesicle were atrophic. B. melitensis was isolated from the spleen (∞ colonies), urine and left femoral bonemarrow. The agglutination reactions with the serum were as follows: B. melitensis 27, 1:200; B. abortus 1:100; B. melitensis 7 and 9, 1:20-40; after absorption with B. abortus 80, the serum agglutinated B. melitensis 1:100.

Guinea-Pig 881.—Was inoculated intraperitoneally with 2 c c of a spleen emulsion of guinea-pig 840. When sacrificed on the 103rd day, the normal spleen weighed 0.91 gm.; the lymphnodes were slightly enlarged, and the uterus presented an endometritis. B. melitensis was isolated from the spleen (31 colonies). The serum agglutinated B. melitensis 11, 1:400, after absorption 1:160.

EXPERIMENTS WITH B. MELITENSIS 29

The strain was obtained in 1919 from Dr. Guido Isar, Catania, Sicily, and was originally isolated from a case of human undulant fever. Five guinea-pigs were inoculated intratesticularly. Four animals were found free from anatomic lesions and gave negative agglutination reactions when sacrificed 72 days after the inoculation. The history of one animal (1001) is presented:

Guinea-Pig 1001.—Weighing 515 gm., was injected into the right testicle with 1/5 slant of B. melitensis 29. The animal presented a rough coat and gave a positive skin reaction. It was sacrificed on the 72nd day after the inoculation, when it weighed 515 gm. Necropsy revealed a small spleen (0.62 gm.); enlarged and firm precrural, iliac and peri-aortic lymphnodes (size of small peas); the left seminal vesicle contained watery pus; the right testis was atrophic and fibrous; the right radiocarpal region was enlarged and showed the typical fibrous thickening about the joint. B. melitensis was isolated from the urine, seminal vesicle, spleen and radiocarpal region. The serum agglutinated B. melitensis 29, 1:400; B. abortus 80.

TABLE 3

Experiments with B. Melitensis 27, Series 2

Guinea-Pig Number	Injected With	Weight		Sacrificed	Anatomic Lesions	Bacteriologic Results	Serologic Findings Serum Agglutinates
		Initial, Gm.	At Necropsy, Gm.				
895	1/25 slant	393	270	Died 10th day	Lung edema, hemorrhagic orchitis	B. melitensis from spleen, testis, but not heart blood	Not tested
896	1/25 slant	389	470	70th day	Spleen 1.8 gm.; distinctly nodular several lymphnodes enlarged. Testis atrophic	Few B. melitensis from spleen	B. melitensis 1:400; B. abortus 80, 1:200 absorbed; B. melitensis 1:100, B. abortus <1:40
897	1/25 slant	424	507	70th day	Spleen 1.2 (3.2 × 1.8 cm.) two nodules; lymphnodes enlarged	Few B. melitensis colonies from spleen	B. melitensis 27, 1:600; B. abortus 80, 1:400; absorbed: B. melitensis 1:100; B. abortus <1:40
898	1/25 slant	443	280	Died 9th day	Right testis necrotic; numerous foci in liver and spleen. Intoxication	B. melitensis from spleen, liver and testis	Not tested

The essential facts dealing with the number of guinea-pigs used for each strain, the course of the infection, the anatomic lesions noted at necropsy and the cultural and serologic results are summarized in table 4. It is shown that 12 strains of B. melitensis inoculated by the intratesticular or intraperitoneal route in amounts varying from 2,000-3,000 millions failed to produce a definite disease, which could be identified either by anatomic lesions or by positive cultures. On the other hand, 4 cultures (11, 21, 26 and 27) which were of human or caprine origin proved highly pathogenic for guinea-pigs, while 6 other strains (6, 19, 18, 20, 22 and 29) and a mixture of 5 strains (2, 3, 4, 5 and 11) occasionally caused lesions.

TABLE 4

SUMMARY OF EXPERIMENTS WITH STRAINS OF B. MELITENSIS

Strains	Number of Guinea-Pig Inoculated	Number of Infections	Course of Infection		Anatomic Lesions						Cultures	Serum Reactions
			Acute and Fatal	Sub-acute and Chronic	Lymph-nodes	Spleen	Liver	Lung	Testis Abscesses	Bones		
1, 2, 3, 4, 5, 7, 8, 9, 10, 23, 24 and 25....	60	0	—	—	—	—	—	—	—	—	—	—
2, 3, 4, 5 and 11...........	41	21	7	14 (44-155)	12	14	9	1	3	—	21	14
6 (Algiers human ?)...	6	2	2	—	2	2	2	—	—	—	2	+2
11 (English human 1896)	31	11	6	5	4	2	1	0	1	0	11	-4
18 (England human 1915)	7	6	4	2	1	2	0	0	1	0	6	+2
19 (England goat).......	8	3	2	1	0	0	0	0	1	0	3	1
20 (Austria human ?)...	8	1	1	—	—	—	—	—	—	—	1	—
21 (England human 1904)	13	9	2	7	5	5	0	1	2	4	9	7
22 (Para melitensis)......	7	5	4	1	1	—	1	—	1	—	5	1
26 (England human 1918)	9	9	2	7	2	4	2	—	4	1	9	5
27 (England goat 1917)..	8	8	2	6	6	6	1	0	(2)	0	8	4
29 (Sicily human ?)...	5	1	0	1	1	0	0	0	1	1	1	—
	203	76	32	44	34	35	16	2	14 (2)	6	76	40

SYMPTOMS AND COURSE OF INFECTION

The testicle, which had been inoculated with the bacterial suspension, became quickly swollen and tender, and the scrotum developed a purple reddish color. In a few animals, local ulcerations were noted. As a rule, the guinea-pigs refused their food for several days, were visibly sick and lost from 100-200 gm. of weight. The temperature was elevated in most instances and varied between 38-40.2 C. Muscular weakness, ruffled coat and tonic spasms usually preceded death from an acute infection. The train of symptoms and the postmortem findings in the 32 instances differed in no way from those described by Eyre,[8] following intracranial injections. Severe intoxications were common in animals infected by the intraperitoneal route. Thirty-two of the 76 successfully infected guinea-pigs died in from 2-20 days. As the experiments reported in this paper were primarily undertaken for the study of subacute and chronic lesions similar to those seen in guinea-pigs infected with B. abortus, all the animals which died in the first 20 days after the injection presented lesions of relatively little interest and are in this presentation classified as having acute, fatal infections. Guinea-pigs suffering from subacute or chronic B. melitensis infections manifested in the first few days after the injection certain of the symptoms already described. The loss in weight was distinct, and later in the course of the disease a rough coated appearance was frequently present, particularly in animals with marked focal lesions. Swelling of the radiocarpal and knee joints was observed in a series of guinea-pigs inoculated with strain 21 and in one animal infected with strain 29.

As regards the course of the disease, the animals divided themselves into 4 groups. Some ran a gradually downward course, became much emaciated and died between the 25th and 50th day after the injection. Larger animals regained their loss slowly and, when sacrificed on the 79th to the 119th day after the injection of 1/10-1/25th of a slant, had nearly reached the original weight, while others registered a loss of from 18-179 gm. In a small number of guinea-pigs, the normal physiologic increase in weight continued, and the animals were disposed of at the end of from 70 to 155 days, in excellent condition. The course of the infection was acute and fatal in 42% and subacute or chronic in 58% of the successfully infected guinea-pigs. Only 76 of the 143 animals infected with 10 different strains presented evidence, either in the form of positive skin tests or in macroscopic and microscopic lesions and positive cultures, which could be considered indicative of an infection. It is evident from these results that certain guinea-pigs have a greater resistance to B. melitensis than others; in fact, some of the experiments even suggested that the animals procured from certain breeders were nonsusceptible to a B. melitensis infection. This is the only apparent explanation which can be offered for the many failures which were obtained in several experimental series. In connection with the experiments on B. abortus, more attention will be paid to this phase of the problem.

Of the 44 animals with chronic infections, 7, or 15.9%, died. Death was due to anaphylaxis in one, enteritis in another, and in the remaining 5 it followed emaciation and chronic intoxication.

PATHOLOGIC ANATOMY

The 32 animals which died in from 2-20 days following the intratesticular or intraperitoneal injection of various strains of B. melitensis, revealed either a necrotizing orchitis or a localized or diffuse fibrinopurulent peritonitis. The liver, spleen and kidneys were dark and engorged with blood; as a rule, the lymphnodes were enlarged and soft. In a few instances, small scattered necroses were visible in the liver. Invariably the inoculated bacteria were isolated from every organ, occasionally also from the heart blood.

The gross pathologic changes in the 44 guinea-pigs, which lived from 47 to 155 days, were in brief:

The lymphnodes were enlarged in 34 cases, or 77.2%. In about one-half of this number, the enlargement was confined to certain groups of nodes; thus the precrural and iliac groups were often quite conspicuous in the anemic inguinal and retroperitoneal fat layers. The firm and grayish nodes varied in size from that of a small grain to that of a small split pea.

The spleen was enlarged in 35 cases, or 79.5%. It should, however, be emphasized that the organ rarely attained the dimensions which were customarily observed in guinea-pigs infected with B. abortus. The average weight of the spleen of 17 infected and carefully weighed guinea-pigs, which lived for 70-155 days, was 1.7 gm. (average body weight 532 gm.). The maximum weight of the spleen was 3.5 gm., for an animal of 417 gm. Not infrequently the dimensions of the organ appeared normal, and without cultural or serologic tests the existence of an infection would have been overlooked. The appearance of the surface under the capsule was always nodular and speckled with grayish foci varying considerably in size, although large whitish areas, as seen in B. abortus spleens, were not observed in this experimental series. When definitely enlarged, the spleen was always darker than normal and presented a moist protruding pulp, with distinct foci on section. In 9 guinea-pigs, even though the spleen presented an apparently normal appearance, definite lesions were found in the genital organs or the ribs.

The liver in 16 guinea-pigs, or 36.6%, showed scattered over the surface either a few or many grayish, pin point foci. These lesions differed in no respect from those noted in guinea-pigs infected with B. abortus. In 3 guinea-pigs, which died on the 25th, the 28th and the 57th day, respectively, the gall-bladder contained a small amount of blood tinged bile, which was teeming with B. melitensis.

Only one guinea-pig revealed grayish foci in both kidneys on stripping the capsule; the genital organs were free from suppurative process in this case. The testes of all animals injected by the intratesticular route showed changes in the form of atrophy and fibrous degeneration of the parenchyma. In 9 instances the organs contained small or large abscesses. The diseased testicle was invariably adherent to the scrotal sac. The epididymis of the testicle into which the bacterial growth had been injected presented abscesses in 2 cases, while the opposite organ was involved in one instance. The seminal vesicle of the infected right testicle contained creamy or thin liquid pus in 6 cases. In 3 instances, the left seminal vesicle was diseased and attached to a slightly atrophic and fibrous epididymis and testicle. The injections were always made in the right testicles. A striking bilateral atrophy of the testes was recorded in 9 animals.

The placenta of a guinea-pig with a triple pregnancy showed flakes of purulent exudate, and B. melitensis was isolated in culture from this material. Another pig with splenic lesions exhibited a chronic endometritis, but gave only a positive culture from the spleen.

The lungs of 2 guinea-pigs, or 4.5%, showed subpleural, translucent foci in the principal lobes. Five guinea-pigs inoculated with strain 21 presented marked swellings of the osteochondral junctions of several ribs. One animal had an involvement of the knee region and another, infected with strain 29, exhibited an enlargement of the right radiocarpal region. The lesions were identical with those described by Fabyean[14] for guinea-pigs suffering from abortion disease.

The histologic changes were, in accordance with the macroscopic changes, either extensive or slight and could sometimes be found only after careful search of numerous sections. Common to all focal lesions in the lymphnodes, spleen and liver were the nests of epithelioidal and scattered giant cells, frequently accompanied by a marked connective tissue hyperplasia. The pathologic process is indistinguishable from that seen in guinea-pigs infected with B. abortus.

Cultural Results

Systematic preparations of cultures disclosed an interesting distribution of B. melitensis in the infected guinea-pigs. Positive cultures were obtained from:

Spleen	33 animals
Testicles, abscesses in epididymis and pus in seminal vesicles	14 animals
Bonemarrow of left or right femur	6 animals
Kidneys and bile	5 animals
Precrural lymphnodes and liver	4 animals
Bone lesions	4 animals
Urine	3 animals
Placenta	1 animal

It will be noted that the spleen furnished positive cultures more frequently than any other organ. In this connection, it should be stated that the number of colonies which grew on the blood plates were in many cases less than 10. Such cultures were often obtained from animals which were sacrificed after a period of observation of more than 100 days. When B. melitensis could not be isolated from the spleen, either the suppurative processes in the genital organs or the bonemarrow and lymphnodes furnished positive cultures. The urine gave cultures of B. melitensis only in 3 instances. These findings are in contrast to those of Durham, of Eyre and of Nicolle and Conseil, who obtained a fairly good growth from the secretion in about 50% of the animals dying from a chronic infection. The cultures procured from the tissues were readily classified by agglutination tests, and, as far as these methods permit correct deductions, it can be concluded that only descendants of the injected strains were recovered. One culture obtained from guinea-pig 655 was tested on a monkey and proved to be a typical strain of B. melitensis.

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[14] Jour. Med. Res., 1912, 26, p. 441.

SEROLOGIC RESULTS

The experiments dealing with this phase of the problem have been reported in paper II of this series. The serum of the infected guinea-pigs agglutinated in a dilution of 1:200-1:2,000 those cultures belonging to the same group of B. melitensis as that used for the infection. As a rule, the reactions were distinct and of diagnostic value. Animals exhibiting no gross lesions and no positive cultures failed to agglutinate either B. melitensis or B. abortus in dilutions above 1:40. Serum tests may give results indicative of an infection, if performed shortly after specific antigens have been used for skin tests. Most of the serums agglutinated B. abortus; in fact, in some instances the latter organism was clumped in higher dilutions than the infecting B. melitensis strain. Absorption tests, however, invariably removed the coagglutinins and thus established the true nature of the infection.

GENERAL AND LOCAL HYPERSENSITIVENESS

The intracutaneous injection of bacterial proteins prepared from B. abortus and B. melitensis cultures produced in the infected guinea-pigs marked skin reactions; in one animal (655) death followed the introduction of the protein preparations in less than 24 hours. Anaphylactic symptoms were noted and lesions of an acute intoxication were present in the form of congestion and small hemorrhages in the viscera.

It was impossible to diagnose the type of infection with the aid of the skin tests. "Aborto" as well as Melitensis protein" produced indurated areolae varying in size from 1.4-1.8 cm., although the existence of disease was definitely indicated in the animals which were not emaciated. Guinea-pigs with local suppurative processes in the seminal vesicles or testicles responded by small indurations; while generalized infections were conducive to large and pronounced skin reactions. Cutaneous hypersensitiveness tests have proved invaluable in segregating the experimental animals. The method deserves more frequent application than it has received hitherto.

SUMMARY

The data presented in the preceding paragraphs indicate that some strains of B. melitensis are capable of causing lesions in guinea-pigs, which are sometimes remarkably similar to those produced by B. abortus in this species of animals. Four cultures, 2 old strains isolated from human cases of undulant fever in 1896 and 1904; 1 human strain isolated in 1915 and 1 caprine strain recovered from goat's milk in 1917 infected guinea-pigs fairly regularly, while 18 other strains either proved nonpathogenic or occasionally produced lesions in the spleen, lymphnodes, etc. It is noteworthy that strain 20, which, morphologically, biochemically, and serologically cannot be separated from the B. abortus group, caused acute infections, but thus far no chronic lesions have been observed. The pathogenicity of B. melitensis strains for guinea-pigs is influenced neither by the origin nor the age of the culture. The experiences collected in this paper suggest that the capacity of this organism to infect guinea-pigs is a property possessed by only a few strains, although the observations of previous workers strongly indicate that recently isolated cultures or body fluids containing B. melitensis will usually produce distinct lesions. Individual susceptibility of the guinea-pigs may be an important factor. On 2 occasions, only 1 guinea-pig of 8 injected with cultures which had been recently isolated from definite lesions developed a chronic infection, while animals procured from another breeder and inoculated with the same strain became diseased and exhibited interesting rib lesions in 5 out of 6 cases. The tissues most frequently attacked are the spleen and lymphnodes. Lesions in the liver, lungs, kidneys and bones have also been observed. It should be emphasized, however, that the pathologic processes in the spleen and lymphnodes of B. melitensis guinea-pigs appear less striking than those seen in this laboratory in animals infected with B. abortus. In fact, the impression has been gained that B. melitensis strains are less invasive for guinea-pigs than B. abortus. It is also recalled that the first series of intraperitoneal infections showed at necropsy perfectly normal viscera, which negative results prompted subsequent use of the intratesticular route of inoculation. Sufficient data are now available to justify the conclusions that in all probability injection by the intraperitoneal or subcutaneous route, or even feeding infections, may cause specific and characteristic lesions, provided certain strains and susceptible guinea-pigs are chosen. Recently isolated strains and particularly cultures or milk specimens procured from infected goats should be tested on guinea-pigs to determine their pathogenic properties.

EXPERIMENTS WITH B. ABORTUS

Since Theobald Smith and Marshall Fabyean[15] called attention to the lesions produced in guinea-pigs after injections with B. abortus, several workers have studied this interesting inoculation disease. The publications of Fabyean,[14] Schroeder and Cotton,[16] Fleischner and Meyer[17] and Smillie[18] recorded definite gross changes in the viscera, enlarged, nodular spleens, lymphadenopathy, atrophy of the testicles, induration and suppuration of the epididymis, minute foci in the liver, etc. In the majority of instances, these lesions followed the injection of milk, infected cotyledons or cultures recently isolated from diseased guinea-pigs. After careful perusal of the American reports, one cannot escape the impression that splenic lesions are always present and that positive infections occur with remarkable regularity, provided the material inoculated contains a sufficiently large number of abortion bacilli. Many years ago one of us (K. F. M.), experimenting with old stock cultures of B. abortus, repeatedly failed to infect guinea-pigs, and for this reason the literature was carefully scrutinized for information which might explain the failures. As some of these references are nearly inaccessible, an attempt is here made to review this data.

[15] Centralbl. f. Bakteriol., 1912, I, 61, p. 549.

[16] Twenty-Eighth Annual Report Bureau of Animal Industry, 1911, p. 139.

[17] Amer. Jour. Dis. Child., 1917, 14, p. 157.

[18] Jour. Exper. Med., 1918, 28, p. 585.

T. C. Evans,[19] of Canada, described in 1914 the lesions occurring after the inoculation of guinea-pigs with infected milk. His description agrees with that of Schroeder and Cotton, with the reservation that the lesions he found were less pronounced. He ascribed the differences to lack of resistance of his guinea-pigs on account of the cold quarters in which they were kept, or the more marked virulence of the Canadian strains. Most of his guinea-pigs died from the infection, which must, therefore, have been virulent, as most workers have found that death is not a usual sequel.

Krage[20] reported, in 1913, that he had inoculated 36 guinea-pigs subcutaneously, intraperitoneally and intramuscularly with 0.5-2.0 c c of a culture of B. abortus, which was virulent for mice. One animal died from peritonitis; 8 succumbed to intercurrent infections. The remainder were sacrificed at the end of 8 months following the infection. No macroscopic lesions were found, but the serum of the animals agglutinated B. abortus in a dilution of 1 : 1,000. In another series of 16 guinea-pigs, he injected 4 strains, highly virulent for mice, subcutaneously and intraperitoneally. Three animals died on the second day and 3 during the second and third month; one presented a hemorrhagic metritis. The remaining 10 were sacrificed at the end of 6 months. B. abortus was isolated from the precrural lymphnode of an intraperitoneally injected animal; the serum agglutinated B. abortus in a dilution 1 : 4,000. Two other guinea-pigs gave positive cultures from subcutaneous abscesses; one of these animals presented a slightly enlarged spleen. The serum of both guinea-pigs agglutinated B. abortus in a dilution of 1 : 100-1 : 400. The serum agglutination titer of the 7 guinea-pigs, which furnished no cultures, varied from 1 : 40-1 : 200.

In a third series, he injected subcutaneously and intraperitoneally the milk of goats, which had been artificially infected and which contained culturally B. abortus. Two died on the fourth day from peritonitis; 10 succumbed to intercurrent infections and only 4 of the remaining 26, at the end of from 5-6 months, gave serum reactions from 1 : 100-1 : 400. Tissue cultures were entirely negative.

Thomsen[21] reviewed, in 1915, the observations which had been made by Holth, in C. O. Jensen's laboratory in Copenhagen. In 1912, Holth injected subcutaneously 2 guinea-pigs, each with 1 and 2.5 c.c. of an agar slant of recently isolated strains of B. abortus. Two animals, sacrificed on the 44th day after inoculation, presented subcutaneous abscesses, which contained bacterioscopically and culturally B. abortus in pure form. One animal presented an enlarged nodular spleen and typical lesions in the ribs, while the viscera of the other guinea-pig appeared normal. The third animal, which died from pneumonia on the 264th day, and the fourth, sacrificed on the 296th day, revealed normal organs at necropsy. In a second series, he injected into 2 guinea-pigs ½ of an agar slant of an old laboratory strain subcutaneously and intraperitoneally; a third animal received ¼ of a slant intraperitoneally. The first guinea-pig died with a loss in weight of 90 gm. at the end of 14 days and exhibited numerous abscesses in the peritoneal cavity, testicles, etc.; the second guinea-pig died on the 11th day with a loss of 190 gm. in weight; it had diarrhea, an enlarged spleen and testicles; a pronounced infiltration was noted at the site of the inoculation. The third guinea-pig died on the 36th day in an extremely emaciated state (loss in weight 320 gm.); necropsy revealed abscesses in the omentum, on the surface of the liver and in the testicles; numerous ribs and both carpal joints showed typical swellings.

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[19] Report of the Director General for the Canadian Veterinary Service, 1914. No. 5 and No. 6, Reports of the Director of Veterinary Research, Union of South Africa, Pretoria, 1919, p. 357.

[20] Centralbl. f. Bakteriol., Ref., 1913, 57, p. 304.

[21] Maanedsskrift for Dyrlaeger, 1915, 27, pp. 13 and 16.

Thomsen, in 1912, inoculated 13 guinea-pigs with fresh milk procured from cows which had aborted and which also gave positive serum reactions for B. abortus. Two guinea-pigs of this series gave cultures for B. abortus from a subcutaneous abscess in one and a splenic nodule in another.

In 1919, Robinson,[22] in South Africa, detailed his studies on about 200 guinea-pigs, which were inoculated with infected milk or other material and killed at different periods after inoculation, varying from 4 weeks to 7 months. He found that only an enlargement of the spleen occurred with any degree of constancy—liver lesions were seen in only 3 cases. No changes were noted in the bones. Furthermore, Robinson concluded that the agglutination test applied to the serums of infected guinea-pigs is of very great use, particularly when the spleen is only slightly, if at all, enlarged and not abnormal in appearance. In his experience it seemed advisable to determine the question of infection in guinea-pigs by a combined study of the macroscopic lesions, the histologic examination and the agglutination test.

Zeller[23] injected guinea-pigs and rabbits intravenously, intraperitoneally and subcutaneously with large doses of B. abortus and B. melitensis. The necropsy findings of the animals sacrificed after varying time intervals were mostly negative. In a few cases, an enlargement of the spleen and mesenteric lymph-nodes was noted, and the injected bacteria were isolated from the spleen, uterus, testicles, urine, milk and, occasionally, the kidneys.

The findings of Krage, Holth and Zeller deserve consideration in a comparative study of the pathogenicity of B. abortus and B. melitensis. In fact, their results may explain some of the failures to produce gross lesions in guinea-pigs injected with B. melitensis cultures. Sufficient data had been collected in this laboratory to confirm the work of T. Smith and Fabyean, Schroeder and Cotton and others on B. abortus infections following the injection of infected milk or tissues of diseased guinea-pigs. Little was known, however, with regard to the effect of injecting old and recent stock cultures. According to the reports of T. Smith and Fabyean, cultures appear equally as virulent for guinea-pigs as infected material derived from cattle. Their tests were limited, however, to 2 old stock cultures, using 11 guinea-pigs. For comparison, these observations were not considered sufficiently comprehensive and a series of experiments was therefore undertaken (1) to compare the pathogenicity of old B. abortus stock cultures with that of B. melitensis cultures when applied by the subcutaneous or by the intratesticular route; (2) to study the lesions produced by recently isolated strains, and (3) to correlate the data collected from studies I and II with the findings on guinea-pigs which had been

[22] No. 5 and No. 6, Reports of the Director of Veterinary Research, Union of South Africa, Pretoria, 1919, p. 357.

[23] Berl. tierärztl. Wchnschr., 1920, 36, p. 345.

TABLE 5

EXPERIMENTS WITH B. ABORTUS 80, SERIES 3

Guinea-Pig Number	Injected With	Weight		Sacrificed	Anatomic Lesions	Bacteriologic Results	Serologic Findings
		Initial, Gm.	At Necropsy, Gm.				
630	1/10 slant	722	642	15th day (moribund)	Lymphadenitis; splenic tumor 2.2 gm.; foci in liver, orchitis	Spleen, liver, testis gave positive cultures	B. abortus, 1:400; B. melitensis 11, 1:80
631	1/10 slant	730	533	23d day (moribund)	Spleen 3.1 gm.; lymphadenitis; foci in liver and lungs, cholecystitis, lesions in numerous ribs; conjunctivitis and opaque cornea	Spleen, bile, liver, urine, lymphnodes, bonemarrow	B. abortus, 1:2000; B. melitensis 5, 1:1000
632	1/10 slant	740	547	44th day, died in hypersensitiveness test	Typical lesions; spleen 4.5 gm.	Spleen, liver, but not blood.....	Not tested
633	1/10 slant	709	465	90th day	Spleen 0.6 gm.; one rib junction right side enlarged; slight lymphadenitis; testes atrophic	Only rib lesion....................	B. abortus 80, 1:200; B. melitensis 11, 1:200
634	1/10 slant	825	797	183d day, negative skin reaction	Spleen small, 0.5 gm.; right testis atrophic	Sterile organs	B. abortus, 1:40; B. melitensis, <1:10
635	1/10 slant	600	292	Died 24th day	Toxemia; spleen, 1.4 gm.; lymphnodes hemorrhagic	Every organ, except heart blood	Not tested

injected with milk or other infected material. These experiments are briefly described in subsequent paragraphs.

INTRATESTICULAR AND SUBCUTANEOUS INOCULATIONS OF STOCK CULTURES OF B. ABORTUS

EXPERIMENTS WITH B. ABORTUS 80

(A) Six guinea-pigs were inoculated intratesticularly with 0.1 c c of an agar slant of a young culture of B. abortus 80; the strain was originally isolated from certified milk and was about 2 years old. The results obtained in this experiment are shown in table 5.

(B) Four guinea-pigs were inoculated subcutaneously with the same amount and suspension of B. abortus 80, as series A. The results were briefly as follows:

Guinea-Pig 802.—Weighing originally 580 gm., gave positive skin tests and, when sacrificed on the 87th day, weighed 570 gm.; the spleen (3.5 gm.) was nodular; the lymphnodes were small, but firm; few foci were noted in the liver. The cultures made from the spleen and liver were negative, but the serum agglutinated B. abortus in a dilution of 1:1,000.

Guinea-Pig 803.—Weighing 612 gm.; the skin tests were positive. When sacrificed on the 87th day, the animal weighed 512 gm. and had a small spleen, 0.9 gm.; the lymphnodes were small and soft. B. abortus was isolated from a small abscess in the right uterine horn and the lateral ligament. The blood serum agglutinated B. abortus in a dilution of 1:600.

Guinea-Pig 804.—Weighing 511 gm., and gave positive skin reactions. It was sacrificed on the 87th day, when it weighed 485 gm. The spleen was small and weighed 1.2 gm. All the organs appeared normal, and the cultures remained sterile. The serum agglutinated B. abortus in a dilution of 1:1,000.

Guinea-Pig 805.—Weighing 570 gm. and, when sacrificed, 521 gm. (87th day). The spleen was small, 0.6 gm. From the uterus, showing an endometritis with a retained, necrotized placenta, B. abortus was isolated. The serum agglutinated B. abortus in a dilution of 1:2,000.

EXPERIMENTS WITH B. ABORTUS 38

The strain was obtained from the Laboratory of the Pennsylvania State Live Stock Sanitary Board; nothing definite is known regarding its origin. Six guinea-pigs were inoculated intratesticularly with 1/5 slant of a young agar culture. The results are presented in table 6.

EXPERIMENTS WITH B. ABORTUS 14

The strain was a stock culture originally obtained from Dr. A. Eichhorn, who had isolated the culture from the uterine exudate of an artificially infected cow. Three guinea-pigs were inoculated intratesticularly with 1/5 slant of a young culture. Two animals (696 and 697) were repeatedly tested with "aborto"-protein, but no reactions were obtained. Both guinea-pigs gained in weight and when sacrificed

TABLE 6

Experiments with B. Abortus 38, Series 1 [*]

Guinea-Pig Number	Injected With	Weight		Sacrificed	Anatomic Lesions	Bacteriologic Results	Serologic Findings
		Initial, Gm.	At Necropsy, Gm.				
679	1/5 slant	249	190	15th day, died	Abscess in right testis; general fibrinous peritonitis	Testis and peritoneal fluid......	Not tested
680	1/5 slant	269	353	74th day, positive skin reaction	Typical abortion disease; spleen 2.4 gm.; foci in liver and kidneys	Spleen, liver, kidneys, urine, positive	B. abortus, 1:1000; B. melitensis 11, 1:600
681	1/5 slant	245	190	Died, 7th day	Hemorrhagic orchitis, splenic tumor, toxemia (?)	Spleen positive; other organs not tested	Not tested
682	1/5 slant	266	248	Died, 54th day positive skin reaction	General lymphadenitis; spleen small (0.6 gm.); other tissues negative	Spleen only tested and found sterile	Not tested
683	1/5 slant	305	420	95th day, positive skin reaction	Spleen small (0.8 gm.); lymphnodes firm, but small; testes atrophic	Spleen ∞ growth..................	B. abortus 80, 1:800
684	1/5 slant	395	267	19th day (moribund)	Necrotized right testis; spleen 0.4 gm.; slight lymphadenitis	Testis, extensive growth.........	Not tested

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[*] Two guinea pigs—701 and 702—inoculated intratesticularly with an emulsion of the testis of guinea-pig 684 developed abortion disease with slight lesions, positive skin reaction, but negative spleen cultures.

on the 99th day presented normal and sterile organs. One guinea-pig (695) became infected. The history is given.

Guinea-Pig 695.—Weighing 460 gm., received intratesticularly 1/5 slant of B. abortus 14. Skin tests on the 44th day were positive. At necropsy (weight 292 gm.) on the 50th day, the spleen was small (0.7 gm.); the lymphnodes were soft and small. The right testis contained an abscess the size of a pea and the left epididymis exhibited several small abscesses. B. abortus was isolated from the pus of the abscesses, but not from the spleen, liver or urine. The serum agglutinated B. abortus 80 in a dilution of 1:2,000 and B. melitensis 11, 1:800.

EXPERIMENTS WITH B. ABORTUS 1

The culture was an old stock culture and had originally been obtained from Sir S. Stockman, who had isolated the strain from the uterine exudate of an artificially infected cow. Three guinea-pigs were inoculated intratesticularly with 1/5 slant of a young culture. Two animals (699 and 700) gave negative skin reactions and, when sacrificed on the 86th day, presented normal and sterile organs, while, according to the history given, one guinea-pig (698) became infected.

Guinea-Pig 698.—Weighing 565 gm., received intratesticularly 1/5 slant of B. abortus 1. Skin tests on the 44th and 66th day were positive. The guinea-pig was chloroformed on the 86th day after the inoculation, when it weighed 570 gm. The spleen was slightly enlarged (weight 1.3 gm.) and nodular; the liver showed a few scars, and the testes were atrophic. B. abortus was isolated from the kidneys, but not from the spleen, bonemarrow, liver, etc. The serum agglutinated B. abortus 80, 1:800.

These experiments indicate that intratesticular injections of B. abortus may produce an acute and fatal disease. Extreme emaciation and exhaustion combined with extensive inflammatory processes are probably the main causes of death. Every guinea-pig injected with strain 38 became infected, but the anatomic changes were only typical in one animal (680). Strain 80 acted similarly; one guinea-pig presented a small spleen when sacrificed on the 90th day, but showed changes in the ribs and testicles. Another animal gave negative skin tests repeatedly and was normal at necropsy on the 183rd day. Strains 1 and 14, probably old stock cultures, are less virulent than strains 80 and 38. The animals survived the injection, but only one guinea-pig of each series gave a positive skin reaction and presented distinct anatomic lesions. As far as the pathogenicity for guinea-pigs is concerned, the effect of these 2 strains cannot be distinguished from that of typical B. melitensis strains. In fact, the gross lesions, the cultural findings and the serologic results were indistinguishable from those previously noted in guinea-pigs inoculated with B. melitensis strains 11 or *27*. Subcutaneous injections of strain 80 gave positive infections. but in only 2 animals was the pathologic picture typical.

The differences in pathogenicity cannot be ascribed to the amounts of culture injected. Young cultures were suspended in saline and the turbidity of the emulsion was standardized against a typhoid vaccine containing 1 billion bacteria per c c. One tenth or 1/5 of an agar slant of B. abortus contained about 1,000-2,000 million viable organisms and was approximately the same for each of the 4 strains. It is naturally impossible to make conclusive statements with regard to the virulence of a B. abortus strain until further knowledge relative to the individual susceptibility of the guinea-pigs has been accumulated, but the few carefully controlled observations rather forcibly suggest that old stock cultures are not only less toxic, but also less invasive than recently isolated strains or infected material derived from bovine, porcine, or cavian sources. This contention is supported by some tests conducted with a strain of B. abortus isolated from a case of swine abortion.

INTRAPERITONEAL INJECTIONS OF B. ABORTUS CULTURES OF PORCINE ORIGIN

In 1920, Dr. J. Traum furnished the writers with a culture of B. abortus isolated from the liver of an aborted swine fetus. The strain grew profusely, in contrast with the bovine B. abortus strains, but could not be distinguished biochemically or serologically from the ordinary stock cultures kept in this laboratory. However, the porcine B. abortus manifested noteworthy invasive properties for monkeys. These facts have already been noted in papers I, II and III of this series. Twenty-four guinea-pigs were inoculated with 1/20 slant of a vigorously growing agar culture of the second generation.

The clinical and necropsy findings are summarized in table 7.

The data presented in table 7 proved the highly virulent character of a porcine strain of B. abortus. Six animals succumbed to the infection in from 17-40 days after the intraperitoneal injection. The loss in weight varied from 73.5 to 345 gm. and only 6 of the 24 animals showed a gain of 10 to 195 gm., or an average increase of 41 gm. The strain exhibited pyogenic properties exemplified by abscess formation in the peritoneal cavity, omentum and lymphnodes, likewise in some instances in the spleen and liver. The testicles, epididymis and seminal vesicles of all the animals used in the test were destroyed by suppurative processes. The spleen was usually enlarged, but 2 animals presented, at least macroscopically, a normal splenic capsule and pulp. Nine animals had involvements of the ribs and long bones, while 11 showed lesions only in the radiocarpal and tibiotarsal regions. The lungs and kidneys were never diseased. B. abortus was readily isolated from the spleen, testicles, etc., and the blood serum agglutinated the representatives of the Brucella group in dilution of 1 : 800-1 : 2,000.

Constant infections with definite localization of the lesions in the sex organs, bone structures, spleen and lymphnodes were not observed with an inoculation of less than 2,000 million organisms. Guinea-pigs purposely procured from several breeders were employed, and the striking results could therefore only be attributed to the inherent properties of the strain and not to the susceptibility of certain animals. Furthermore, F. Hayes and J. Traum [24] and Schroeder [25] have recently reported identical results, using the same and other porcine strains on guinea-pigs obtained from different sources.

TABLE 7

Necropsy Findings on Guinea-Pigs Inoculated with B. Abortus Cultures of Porcine Origin

Died or Sacrificed	Number	Average Loss or Gain in Weight, Gm.	Spleen, Average Weight	Lymphnodes	Liver Abscesses	Abscess in Omentum	Abscess in Testes or Epididymis	Involvement of Ribs	Radiocarpal Tibiotarsal Region
Died, 17 days	2	167	1.3	Abscesses 2	0	2	1 (1 female)	0	0
Died, 18 days	1	112	0.8	Enlarged	0	1	1	1	0
Died, 20 days	1	196	0.9	Small	0	1	1	1 both sides	0
Died, 38-40 days	3	126	1.1	3 enlarged	1	3	2 (1 female)	2	1
Sacrificed 55 days	2	345	3.35	2 enlarged	1	2	1 (1 female)	0	0
Sacrificed 69 days	14	8—73.5 6+41.0	3.57	12 enlarged	9	8	4 (10 females neg.)	5	9
Sacrificed, 134 days	1	—238	2.21	1 enlarged	1	1	1	0	1
	24	.....	...	21 enlarged	12	18	11	9	11

INOCULATIONS OF GUINEA-PIGS WITH INFECTED MILK, GUINEA-PIG TISSUES AND CULTURES OF B. ABORTUS

Since 1915, the writers have been interested in the occurrence of B. abortus in "certified milk" and later in the problem of cutaneous hypersensitiveness in guinea-pigs infected with B. abortus or B. melitensis. In the course of these studies, several hundred male and female guinea-pigs were inoculated with milk sediment, spleen emulsions of infected animals or cultures of B. abortus, etc. The necropsy examinations of 114 animals were conducted according to the principles laid down in the paragraph on methods, and the data, although originally collected for entirely different purposes, can therefore be used in a discussion dealing with the pathogenicity of B. abortus for guinea-pigs. For the sake of brevity results of importance are presented in tables 8 and 9.

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[24] Jour. Amer. Vet. Med. Assn., 1922, 60, p. 435, and personal communication.

[25] Jour. Amer. Vet. Med. Assn., 1922, 60, p. 560.

It is to be regretted that the data summarized in tables 8 and 9 do not lend themselves to a discussion of the relative susceptibility of guinea-pigs to B. abortus, nor do the findings permit any conclusions relative to the virulence of the various strains employed. In the first place, it was obviously impossible to determine the number of viable organisms in the injected tissues or milk, and, secondly, in those instances in which the number of inoculated bacteria was known, namely, not more than 1,000-2,000 million bacteria per dose, it was, for various other reasons, necessary to keep the animals under observation for at least 300-400 days. As early as 1916, it was noted that a series of 48 guinea-pigs injected with old stock cultures isolated in 1910 and 1911 not only survived the infection, but 8 animals failed to give skin reactions and on necropsy were found to be free macroscopically, microscopically and culturally from B. abortus. At that time serum tests were not conducted systematically, and this series can therefore be considered only from the standpoint of gross lesions. The data in tables 8 and 9 have been arranged according to the material which was used for the inoculations. It is quite evident that from the tables it is possible to classify the infections as acute, subacute or chronic. The following facts are apparent:

Guinea-pigs inoculated with infected milk, cultures, etc., and permitted to live for from 29-200 days always exhibited enlarged, firm lymphnodes; the spleen was typical and enlarged (1.2 to 10.5 gm. or an average of 4.69 gm.) in 80% of the cases. Normally sized spleens were more frequently seen among females than males. Animals, which lived from 200-395 days, classified here as chronic infections, had small lymphnodes and normal spleens. Sixteen of 17 males, which came to necropsy on the 300-389th day, presented spleens of an average of 0.83 gm. In many instances, however, this organ was adherent to the abdominal wall and, on section, it was strikingly fibrous and dry. Atrophy of the testicles was always present, while abscess formation in these tissues was found in 12, or 70%, of the animals. In the acute

TABLE 8

B. Abortus Infections in Guinea-Pigs

Male and Female Guinea-Pigs Inoculated with Milk Sediment or Spleen Emulsions of Infected Guinea-Pigs

Day on Which Sacrificed	Number of Guinea-Pig		Lymphnodes				Spleen				Liver		Lungs		Kidneys		Testicles		Uterus		Bone Lesions				Cultures from				Agglutination Titer of Serum	
			En-larged		Nor-mal		En-larged		Nor-mal								Atrophy	Ab-scesses	Preg-nancies	Ab-scesses	Ribs		Joints		Spleen		Ab-scesses		>1:1000	<1:1000
	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.					M.	F.	M.	F.	M.	F.	M.	F.		
29	..	1	..	1	..	..	....	1	..	..	..	1	..	..	..	..	...	...	...	...	..	..	..	..	..	1	..	..	..	1
54	2	2	2	2	—	—	2	1	—	1	..	1	.	..	..	..	...	...	...	...	..	..	..	..	2	2	..	..	4	
60-69	..	2	..	2	..	..	....	1	..	1	..	1	..	..	..	..	...	...	...	1	..	..	..	..	..	1	..	1	2	0
80-89	9	3	9	3	..	..	8	2	1	1	2	2	..	..	..	..	4	...	1	1	..	..	..	..	9	2	..	1	10	2
90-99	14	5	14	3	..	2	14	4	..	1	6	3	..	1	.	..	9	2	2	...	1	..	..	..	14	4	2	0	17	0
100-109	6	3	6	3	..	..	4	2	2	1	1	1	1	1	1	..	4	1	...	...	..	..	..	..	4	2	0	..	6	3
							(1 R.)																							
110-119	..	2	..	2	..	..	....	1	..	1	..	..	..	..	..	..	...	...	1	...	..	..	..	.	..	1	..	..	1	1
120-129	..	2	..	2	..	..	....	1	..	1	..	1	.	1	..	..	0	...	...	...	..	..	..	..	..	1	..	..	2	0
130-139	3	2	3	2	..	..	1	....	2	2	..	2	..	..	..	..	2	...	1	...	..	..	..	..	2	1	..	..	1	4
140-149	4	2	4	2	..	..	3	2	1	..	..	..	2	..	..	..	4	1	...	1	1	..	..	..	2	1	..	1	4	2
150-159	2	..	2	..	..	..	2	....	..	..	1	..	..	..	..	..	2	1	...	...	1	..	..	..	2	..	1	..	1	1
160-169	3	1	3	1	..	..	3	1	..	..	1	1	1	1	.	..	2	2	...	...	..	..	1	..	1	1	2	..	2	2
170-179	3	1	3	1	..	..	1	1	2	..	1	..	..	1	..	..	2	0	...	...	..	..	..	..	1	..	..	..	1	3
180-189	1	..	1	..	..	..	1	....	..	..	..	..	..	..	..	..	...	...	...	...	..	..	..	..	1	..	..	..	1	0
190-199	1	..	1	..	..	..	....	....	1	..	..	..	..	..	..	..	...	...	...	...	..	..	..	..	1	..	..	..	1	0
200-209	2	1	2	1	..	..	1	....	1	1	.	..	..	..	..	..	2	...	...	...	..	..	..	..	..	..	..	..	1	2
	50	27	50	25	..	2	40	17	10	10	12	13	4	5	1	..	31	7	5	3	3	..	1	..	39	17	5	3	54	21

TABLE 9

Chronic B. Abortus Infections in Guinea-Pigs

Male and Female Guinea-Pigs Inoculated with B. Abortus Cultures

Day on Which Sacrificed	Number of Guinea-Pig		Lymphnodes				Spleen				Liver		Lungs		Kidneys		Testicles		Uterus		Bone Lesions				Cultures from				Agglutination Titer of Serum	
			En-larged		Nor-mal		En-larged		Nor-mal								Atrophy	Ab. scesses	Preg-nancies	Ab-scesses	Ribs		Joints		Spleen		Ab-scesses		>1:1000	<1:100
	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.	M.	F.					M.	F.	M.	F.	M.	F.	M.	F.		
38	..	3	—	3	..	..	....	3	..	..	..	3	..	..	..	..	...	...	...	...	..	..	..	..	..	3	..	..	..	3
50-59	1	2	1	2	..	..	1	2	..	..	..	..	..	..	..	..	1	...	...	...	..	..	.	..	1	1	..	..	3	0
62	1	1	1	1	..	..	1	1	..	..	1	1	..	..	1	..	1	...	...	...	..	1	..	1	1	..	1	1	2	0
98	..	1	..	1	..	..	....	(1 R.)	..	..	..	1	..	..	..	..	...	...	...	...	..	..	..	..	..	..	..	..	1	
100	4	3	4	3	..	..	4	....	..	3	2	2	..	..	2	..	4	3	...	1	..	..	..	..	4	2	3	1	1	6
310-319	5	2	..	2	5	..	0	1	5	1	..	1	..	..	..	..	4	3	...	1	..	..	1	1	2	1	3	1	4	3
330-339	2	..	..	..	2	..	0	0	2	..	..	..	..	..	..	..	2	2	...	...	..	..	..	..	..	..	2	0	1	1
340-349	3	..	1	..	2	..	1	....	2	..	1	..	..	..	..	..	3	2	...	...	..	..	2	..	1	..	2	..	1	2
350-359	1	1	..	..	1	1	....	....	1	1	..	..	..	..	..	..	1	...	...	1	..	..	..	..	1	..	—	—	—	2
360-369	4	0	..	0	4	..	....	....	4	..	1	..	..	..	..	..	4	3	...	...	..	..	1	..	1	..	3	..	2	2
380-389	2	..	..	..	2	..	....	....	2	..	..	..	..	..	..	..	2	2	...	...	..	..	..	..	..	..	2	—	1	1
395	..	1	..	..	..	1	....	....	..	1	..	..	..	..	..	..	...	...	...	1	..	..	..	..	..	..	..	1	..	1
	23	14	7	12	16	2	7	8	16	6	5	8	..	..	3	..	22	15	...	4	..	1	4	2	11	7	16	4	16	21

infections, a bilateral atrophy of the sex organs was noted in 60% and definite abscesses in 14%. Liver and lung lesions occurred more often in the course of the subacute disease; in fact, no pathologic processes in these organs were recorded in animals inoculated with cultures. Kidney lesions were rare, and involvement of the bones of the extremities was somewhat more frequently noted in the chronic than in the acute or subacute cases. Blindness was seen in 2 males suffering from a chronic infection. Isolation of B. abortus from the spleen was rarely successful in the chronic infections, but the pus of the focal lesions invariably contained a pure growth of the organism. The serum reactions rarely indicated the age of the disease; numerous guinea-pigs tested on the 100-150th day after inoculation agglutinated B. abortus in dilutions 1 : 200-1 : 400, while others living for more than 300 days clumped the organisms in dilutions as high as 1 : 4,000.

SUMMARY

The course and the character of the lesions produced in 160 guinea-pigs by the inoculation of 1,000-2,000 million organisms of different strains of B. abortus or body fluids or tissues containing this bacterium can be summarized in 3 groups, namely:

(a) Acute toxic and fatal infections frequently follow the intratesticular inoculation of certain stock cultures. In this series, they occurred also following the intraperitoneal injection of a strain of B. abortus isolated from an aborted swine fetus. Lesions in the spleen are slight or absent; B. abortus can be found in all tissues, but not in the heart blood.

(b) The classical subacute infections, as described by T. Smith and Fabyean, with very low mortality, pronounced cutaneous hypersensitiveness, positive serum reactions and distinct lesions in the lymphnodes, spleen, liver, kidneys, lungs, bones, etc., follow quite regularly the subcutaneous, intraperitoneal or intratesticular inoculation of infected material or recently isolated cultures. Old laboratory strains may occasionally produce this form of the disease in a few guinea-pigs. The spleen is always enlarged and nodular, while the other tissues may or may not be affected. Some strains exhibit a tendency to localize in the bone structures.

(c) Chronic infections with cutaneous hypersensitiveness, marked positive serum reactions and small spleens, but definite lesions in the genital organs or bones of the extremities, are noted in guinea-pigs injected with old stock cultures. Male guinea-pigs present at necropsy

a small spleen, abscesses in the testicles, epididymis, or a suppurative seminal vesiculitis and swelling of the radiocarpal or tibiotarsal regions. Cultures can frequently be procured only from the pus of these lesions. Females may fail to show gross changes; they are never pregnant, and they may present a vaginal discharge. The spleen is always fibrous and atrophic, but may occasionally yield a positive culture. The existence of an infection can only be established by skin tests with aborto protein, by serum agglutination tests, or by careful histologic studies.

The average weight of the spleen in the various groups is as follows:

	Average Body Weight	Spleen Weight
Normal guinea-pigs (50)	485.4 gm.	0.79 gm.
Group A—Acute and fatal (12)	376.4 gm.	1.3 gm.
Group B—Subacute (10)	440.0 gm.	4.69 gm.
Group C—Chronic (14)	607.5 gm.	0.83 gm.
B. melitensis infections (17)	532.0 gm.	1.70 gm.

The experiments reported in these paragraphs support the view that B. abortus may produce in guinea-pigs an inoculation disease with tissue injury of varying degree of intensity. This conclusion applies in particular to the changes in the spleen and to the infections produced with cultures. The acute infection, as a rule, extends over a relatively short period and reparative processes appear at the end of a month. These animals may recover but harbor the inoculated B. abortus in foci of the genital organs. This sexotropism, which follows any form of injection, is remarkably constant and is of considerable diagnostic value. Infections involving all the tissues of the body with the exception of the muscles follow the injection of a porcine strain of B. abortus. The enlargement of the spleen has been most striking and constant in guinea-pigs injected with body fluids or tissues infected with bovine B. abortus.

The observations are not sufficiently extensive to warrant final conclusions, but they indicate that old B. abortus cultures may either fail to cause a disease, or the anatomic lesions are so slight that they cannot be seen with the unaided eye. It is this group of cases which resembles anatomically the infections caused by the intratesticular or intraperitoneal injections of the majority of B. melitensis cultures.

DISCUSSION

This comparative study has clearly shown that the two organisms of the Brucella group are pathogenic for guinea-pigs, but B. abortus is, as a rule, slightly more invasive and virulent than B. melitensis. As has been pointed out, this statement deserves further experimental study with B. melitensis strains derived from caprine or human sources. In paper III of this series of investigations, it has been shown that B. melitensis is far more invasive for monkeys than B. abortus, while the tests on guinea-pigs apparently prove the reverse. The organisms which have been tentatively placed, on account of their bacteriologic similarity, into one group, can probably in the light of the studies of this laboratory be distinguished by their serologic behavior and pathogenicity for monkeys and guinea-pigs. For the experimental pathologist this peculiar variability in the pathogenicity for laboratory animals offers many fascinating problems. The solution of the question "Can the B. abortus be transformed into the B. melitensis and vice versa?" deserves careful attention in the future. Some attempts along this line have been made without obtaining any definite leads. Furthermore, cross-immunization tests have been undertaken with equally negative results. In two series, 5 guinea-pigs were treated for several months with ascending doses of fresh heat killed cultures of B. melitensis 11 and 20, B. paramelitensis 9 and B. abortus 80. Ten days after the last injection, each animal, together with 5 control, normal guinea-pigs, were inoculated subcutaneously with 2,000 million B. abortus strain 80, or strain swine fetus. Certain normal and certain immunized animals died acutely following the injection of the porcine B. abortus strain, while the surviving guinea-pigs, when sacrificed on the 60th-100th day after the test inoculation, presented either moderately severe lesions when injected with B. abortus 80 or extensive tissue changes when tested with the highly invasive porcine strain. The vaccinated guinea-pigs exhibited the same lesions as the control animals. Studies are in progress to solve the same problems on rats and mice.

The anatomic lesions produced in guinea-pigs by the injection of B. melitensis deserve some consideration and may perhaps explain some of the necropsy findings reported in human and caprine cases of undulant fever. As the mortality from this disease is usually low, the number of carefully conducted human postmortems is relatively small, and the available information is correspondingly small and incomplete. However, some facts immediately attract attention: Thus, it is stated by Kennedy [26] that the lymphnodes are predominantly diseased, the spleen is enlarged and, according to Basset-Smith,[27] the latter organ shows microscopically a marked increase in endothelial cells and a decrease in lymphatic tissues. Not only the mesenteric lymphnodes, but also the axillary and inguinal nodes are enlarged and contain innumerable specific organisms.

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[26] Report Mediterranean Fever Commission, 1906, Part IV, p. 94.

[27] Jour. Roy. Army Med. Corps, 1908, 10, p. 70.

Fluctuating swellings over the sternum and ribs have sometimes been mistaken for abscesses, but no pus could be procured. In reading these descriptions, one recalls the rib lesions noted in guinea-pigs. The clinical histories of Luger,[28] Lombard and Bégner[29] and others mention epididymitis, orchitis, periostitis and mono-arthritis as metastatic processes, which are not uncommon in the course of an undulant fever attack. Eyre[30] records amenorrhea and occasionally abortion as sequelae of B. melitensis infections in women.

The Reports of the Mediterranean Commission, of Mohler and Hart[31] and of others dealing with the necropsy findings of goats infected with B. melitensis record an enlarged spleen, general enlargement of the lymphnodes, distinct hyperemia of the liver and kidneys. Abortion is a frequent sequel of malta fever in goats. Furthermore, B. melitensis has been isolated regularly from the spleen, lymphnodes, mammary glands and occasionally from the urine. The diseased tissues have not been studied extensively. Spagnolio,[32] who examined the spleens of 42 goats, noted focal nodules consisting of epithelial cells and central necroses, while Neri[33] reported the lesions of a definite mastitis. There is no doubt that B. melitensis possesses in man and goats, just as B. abortus in guinea-pigs, a predilection for the perivascular and subcapsular tissues of the spleen, lymphnodes and bone structures. The specific ubertropism of both organisms is only an expression of a marked sexotropism, which in females may lead to abortion or localization of the bacterium in the udder; while in males it manifests itself in the form of suppurative processes in the testicles, epididymis, etc. The latter fact, known for B. melitensis in man, has recently been recognized by Buck, Creech and Ladson[34] for B. abortus in bulls. It is evident from the observations recorded in this paper and the theoretical considerations just presented that the bacteria belonging to the Brucella group exert their specific pathogenic properties not only in the original host, the goat, the cow and the swine, but also in the small laboratory rodents. It is therefore not unlikely that our knowledge concerning the pathogenesis and immunity, which is intimately connected with the control and eradication of two important economic diseases, namely, infectious abortion in cattle and swine and undulant fever in goats, can probably be advanced by a careful study of the inoculation disease in guinea-pigs.

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[28] Deutsch. med. Wchnschr., 1921, 47, p. 321.

[29] Presse Méd., 1921, 29, p. 753.

[30] Handbuch d. path. Mikroorg., 1912, p. 438.

[31] Twenty-Fifth Annual Report of the Bureau of Animal Industry, 1908, p. 279.

[32] Centralbl. f. Bakteriol., Ref., 1908, 42, p. 677.

[33] Ann. d'ig. sper., 1912, 21, p. 321.

[34] Jour. Agric. Res., 1919, 17, p. 239.

CONCLUSIONS

The experiences collected in this paper indicate that certain strains of B. melitensis are capable of producing an acute, subacute, or chronic inoculation disease in guinea-pigs. The gross anatomic and the histologic changes resemble those commonly noted in guinea-pigs infected with B. abortus. In fact, it is sometimes impossible to distinguish the two infections without careful serologic cross absorption tests. Four cultures, 2 old strains isolated from human cases of undulant fever in 1896 and 1904, one human strain isolated in 1915, and one caprine strain recovered from goat's milk in 1917, infected guinea-pigs regularly, while 18 other strains proved either nonpathogenic or produced in exceptional instances lesions in the spleen and lymphnodes. Aside from the inherent pathogenic property of certain strains, it is not unlikely that the individual susceptibility of the guinea-pigs and the mode of injection are in a large degree responsible for the course and the character of the infection. Intratesticular injections have been used most frequently.

Thirty-four of 44 guinea-pigs, which were sacrificed 44-155 days after the injection with cultures, presented advanced tuberculosis-like lesions in the spleen and lymphnodes. The average weight of the diseased spleen was 1.7 gm. The largest spleen observed in these experiments weighed 3.5 gm. Localization of the disease in the liver and testicles, as well as proliferation of the bone structure, has been recorded. As a whole, the lesions produced by B. melitensis in guinea-pigs are less extensive and severe than those produced by B. abortus.

Experiments extending over several years support the well known fact that B. abortus may produce in guinea-pigs an interesting inoculation disease. However, the degree of tissue injury in the spleen and lymphnodes may vary considerably. Sexotropism, which follows any form of inoculation whether caused by infected material or cultures, is remarkably constant. Infections involving all the tissues of the body, with the exception of the muscles, has been characteristic for a strain of B. abortus isolated from an aborted swine fetus. Enlargement of the spleen with a maximum weight of 10.5 gm. and an average weight of 4.69 gm. has been noted in guinea-pigs injected with infected milk or tissues. Old laboratory cultures, as a rule, produce lesions which are so slight that they cannot be seen with the unaided eye. This group of cases resembles anatomically the infections caused by intratesticular or intraperitoneal injections of certain B. melitensis cultures.

Recently isolated strains and, in particular, cultures or milk specimens procured from goats affected with malta fever should be tested for pathogenic properties on guinea-pigs.

TREASURY DEPARTMENT
UNITED STATES PUBLIC HEALTH SERVICE

PUBLIC HEALTH BULLETIN No. 127
SEPTEMBER, 1922

THE EPIDEMIOLOGY OF BOTULISM

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Prepared by Direction of
THE SURGEON GENERAL

WASHINGTON
GOVERNMENT PRINTING OFFICE
1922

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THE EPIDEMIOLOGY OF BOTULISM.[1]

PART I.

INTRODUCTION.

Impressed by the grave responsibility thrust upon the canning industry of this country incidental to the tragic botulism outbreaks, which followed the consumption of factory-preserved ripe olives, the National Canners Association, the Canners League of California, and the California Olive Association proposed in December, 1919, a detailed investigation in order to determine the danger from botulism, how it arises, and how it can best be avoided and overcome. Through the instrumentality of the above-named associations, funds were provided to support research work on certain definite phases of botulism in the United States, and a commission was formed in California to supervise the work on the Pacific coast. The commission was composed of the following members: J. C. Geiger, epidemiologist of the United States Public Health Service, E. C. Dickson, associate professor of medicine in the Stanford University Medical School, and K. F. Meyer, professor of research medicine and acting director of the George Williams Hooper Foundation for Medical Research, representing the University of California and, as consulting bacteriologist, the California State Board of Health.

It was decided that the investigation should be conducted in three directions: First, a careful statistical analysis of the anatomical and bacteriological data, which were already available or which could be collected from previous outbreaks of human and animal botulism; second, a clinical study of typical and atypical cases with special emphasis on the value of the specific serum treatment; and, third, an extensive experimental study dealing with the distribution and sources of *B. botulinus*, including particularly an investigation of the factors predisposing to the production of toxin in certain types of food. The original plans to confine the study to botulism in the State of California were subsequently broadened and this report includes a study of all the outbreaks in the United States concerning which records were available.

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[1] This investigation was aided by a grant from the National Canners Association, the Canners League of California, and the California Olive Association.

The epidemiologic field studies were undertaken by Dr. J. C. Geiger, who personally investigated every important outbreak of botulism occurring in the States of California, Oregon, Washington, Arizona, and Colorado. Health officers, physicians, and officials of the various food and drug departments were interviewed relative to food poisoning cases resembling botulism. Through the cordial cooperation of various agencies, prominent among which were the western district of the Federal Bureau of Chemistry, various local and State boards of health and many individual physicians and veterinarians, the occurrence of outbreaks was usually early reported to the members of the commission. It should also be mentioned that attention to the possible prevalence of food poisoning was sometimes drawn by casual press reports. It is most unfortunate that food poisoning, food infections, and botulism are reportable diseases only in the States of California, Oregon, New York, and Kansas. Many public-health authorities possess no information relative to the frequency of food or "ptomaine poisoning" and an accidentally recognized case of botulism is either dismissed as unimportant or is not studied in a manner which enables an epidemiologist to reconstruct the important phases of the outbreak. Not infrequently improperly qualified and inexperienced persons are entrusted with the investigation of food-poisoning outbreaks. Evidence of the greatest value is overlooked and the incriminated food is referred to laboratory workers who are not qualified to pass judgment on a food suspected of causing poisoning. It is quite evident that the data analyzed were obtained from a diversity of sources and therefore cannot be of desired unformity. In several instances the facts available are so unsatisfactory that definite conclusions are necessarily impossible.

EPIDEMIOLOGY OF BOTULISM.

No. . . . .
Series: . . . . County: . . . . Town: . . . .

CLINICAL DATA.

Name: . . . . Age: . . . . Sex: . . . .
Residence: . . . . Occupation: . . . . Address: . . . .
Date of onset: . . . . Physician and address: . . . .
. . . . Diagnosis: . . . . Date seen: . . . .
Present status: . . . . Recovery: . . . . Died: . . . .
Symptoms: . . . .
Treatment of fatal and recovered cases: . . . .
. . . . Serum treatment, if any: . . . .
Autopsy: . . . . Date: . . . . If coroner's case, state: . . . .
By whom: . . . .
Anatomical findings: . . . .
Laboratory examinations of clinical and autopsy material: . . . .

EPIDEMIOLOGY.

Causative meal:.. Date:..............
Home or factory canned:.............. How:.......... Date:..............
Approximate amount consumed:..
Causative food factors:.............. Taste:.............. Description:..........
Laboratory examination:..

[Back of card.]

ANIMAL INTOXICATIONS.

Animal on premises:........ Number of horses affected:......... Feed:..........
Number of cattle affected:.......... Feed:..........
Number of hogs affected:.......... Feed:..........
Number of chickens affected:...... Feed:..........
Number of turkeys affected:........ Feed:..........
Date of illness:.............. Symptons:..
Number of deaths:..
Autopsy:................ By whom:...................... Date:..............
Anatomical findings:..
Laboratory examination:..

REMARKS.

....

....

For the collection of the data by the commission a filing card (5 by 8 inches) was prepared, covering the significant points regarding the patients and the causative meal. Space was provided on these cards for a study of botulism in animals, inasmuch as it was sometimes intimately associated with human cases. The details of the card are illustrated above.

Pathologic and bacteriologic studies of food and soil samples were conducted in the laboratories of the University of California and Stanford University. The bacteriologic researches were carried out by Miss Bertha Dubovsky and Miss Florence Field. Studies of the pathologic histology of the material were undertaken by Dr. W. Ophüls and Dr. G. Y. Rusk.

ACKNOWLEDGMENTS.

It is not practicable to make personal acknowledgment to the many health officers, physicians, veterinarians, etc., to whom the commission is under great obligation for interest and cooperation. Patients were approached only through their local physicians and health officers and in no case did the commission find anything but the most ready cooperation on the part of the medical advisers and health authorities, many of whom actively associated themselves in the work at a considerable expenditure of time and effort.

Part II.

GENERAL CONSIDERATIONS.

How frequently botulism occurs in the United States is not definitely known and the statistics on food poisoning fail to suggest even approximately the incidence of this intoxication. Since cases of food poisoning, "ptomaine poisoning," and similar maladies are not legally reportable, public-health authorities possess no information relative to their occurrence. Jordan (1) has shown that inaccurate conclusions can be drawn as to the prevalence of certain kinds of food poisoning by the casual press reports which are available. He collected through a press clipping bureau and other sources during the period October, 1913–October, 1915, a total of 375 group and family outbreaks, which involved 5,238 persons. It is not unlikely that the number ascertained falls far short of the actual status. Jordan (1) reached the conclusion that "probably several thousand outbreaks of food poisoning in families and larger groups, affecting at least 15,000 to 20,000 persons, occur in the United States in the course of a year."

The number of fatal cases of food poisoning varies greatly in different outbreaks and inasmuch as no definite records are available, one is again forced to make use of inconclusive evidence. According to a compilation made by the United States Public Health Service from the mortality statistics in the United States, 5,537 deaths are attributed to poisoning by food from 1910 to 1918, inclusive. It is naturally impossible to verify the diagnoses on which these figures are based, but it is not unlikely that a number of deaths have been and are at present incorrectly considered the result of an attack of food poisoning. In subsequent chapters it will be shown that the case mortality rate in European food poisoning outbreaks is 1.5 per cent. Applying this rate to the American data estimated by Jordan it is noted that the actual mortality from poisoning in the course of one year would approximate 300 deaths. From Chart I, it is, however, evident that the actual mortality from food poisoning according to the above-mentioned statistics has increased from a total of 157 cases in 1910, to a total of 842 cases in 1918. The estimates of Jordan must therefore be looked upon as conservative.

There is little doubt that intestinal or other disorders due to particular articles of food occur more frequently than is generally believed to be the case. As a cause of death food poisoning and food infections are less important than, for example, typhoid fever. In this connection, it is recalled that the actual number of cases of typhoid fever in the United States in the year 1914 was estimated at 198,000 and the deaths were probably 19,800. (Dublin Am. Jour. Public Health, 1915, 5, p. 20.) Judging from the usual newspaper publicity given to food poisoning outbreaks one would conclude that not typhoid fever but food poisoning is a common disease. The tendency to incriminate preserved food as the cause of disease or death is certainly not the outcome of scientific inquiries but rather the result of popular prejudice and tradition.

In Table 1, the figures representing the mortality attributed to food poisoning in several States are compared with those proven to be due to botulism. The latter figures regarding botulism are compiled from the data collected by the commission and are in all probability fairly complete for the period 1915–1921. This statement is particularly true for the deaths which occurred in groups and where there was more than one fatality in a family. These group fatalities, on account of their dramatic character, attract considerable attention, stimulate medico-legal investigations, and lead to publicity in the daily press or in medical literature. Moreover, during the stated period a number of agencies have been interested in botulism and it can be assumed that every group of food intoxications has been carefully scrutinized for botulism cases. On the other hand, it should be remembered that botulism may be mistaken for methyl-alcohol poisoning, epidemic encephalitis, cerebrospinal syphilis, etc. Furthermore, it is not unlikely that quite a number of single fatal cases of true botulism have been diagnosed and reported incorrectly or have been placed, for the sake of convenience, in the all-embracing class of "ptomaine poisoning." If one admits that the mortality statistics on botulism are incorrect or incomplete it is quite obvious that in all probability a number of nonfatal cases have escaped recognition. The records of clinical cases of botulism which occurred in groups and families indicate that the consumption of the poisonous food may frequently produce mild or indefinite symptoms. These incidences of intoxication may be overlooked.

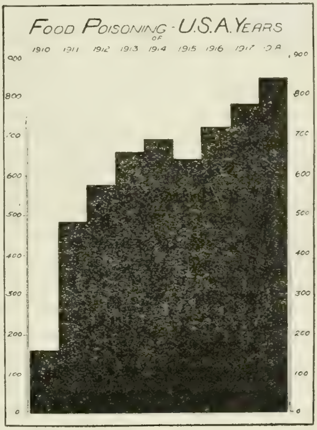


FIG. 1.

Definite information relative to the extent of fatal or nonfatal botulism in the United States can only be expected when all suspected cases are carefully investigated and the clinical diagnosis confirmed by toxicological or bacteriological methods.

TABLE 1.—*Mortality food poisoning in 10 States of North America.*

	1910–1917		Total.	Death from botulism, 1910–July 31, 1921.[1]	
	Cities.	Rural districts.			
California	179	202	381	76	(1910–1918 [1]=59 =15 per cent).
Colorado	25	71	96	13	
Indiana	80	171	251	6	
Massachusetts	130	50	180	4	
Michigan	107	166	273	10	
Montana	17	29	46	5	
New York	269	177	446	16	
Ohio	189	215	404	7	
Pennsylvania	161	276	437	6	
Washington	64	84	148	16	(1910–1918 [1]=8 =5.4 per cent).

[1] Note discrepancy in periods.

Considering the figures presented in Table 1, apropos of the above discussion, it is evident that outbreaks of botulism are not infrequent in the United States. In California and Washington, two States in which the commission has made very careful inquiries relative to the incidence of the intoxication, only 15 and 10.8 per cent respectively of the total mortality due to food poisoning can with certainty be attributed to botulism. In other States this percentage is even smaller, for example in New York, probably less than 4 per cent of all deaths due to food poisoning are presumably caused by this intoxication. As a cause of death, however, this disease is by no means as important as the prominence given recently by the daily press and by numerous publications in the medical journals would imply. Although the number of actual cases of botulism is thus far comparatively small, it should, however, be emphasized that in recent years a striking increase in this disease has taken place in various localities. Foodstuffs which were considered to be unsuitable for the development of *B. botulinus* have recently been found to be the cause of this type of poisoning, and laboratory studies have demonstrated that *B. botulinus* is more widely disseminated in nature than has generally been suspected. All these facts create a feeling of uneasiness.

The question whether botulism is an old or new disease of the United States can not be answered conclusively. A review of the available records reveals the fact that the first clinically characteristic cases were described in the period from 1899 to 1902. The existence of the disease was later definitely established by the publications of Sheppard (2), of Peck (3), and Wilbur and Ophuls (4), which appeared in the period from 1907 to 1914. From the subsequent records in the medical literature one gains the impression that botulism in the United States is an acquisition of the last 5–10 years. It is the purpose of this epidemiological study to determine the factors responsible for the occurrence and furthermore to establish, if possible, the forces operative for its apparent alarming increase. It is obvious that the few questions just raised can only be answered after a discussion of botulism from a clinical, pathological, bacteriological, and toxicological viewpoint. Such a discussion must also consider the causative organism and the various contributory factors inducing the formation in certain foodstuffs of highly potent toxin. Although most of the information relative to the last-named factor can only be obtained through an extensive experimental study, an attempt is herewith made to draw from the reports of the various botulism outbreaks in the United States as much evidence as possible bearing on this point. Inasmuch as the commission concentrated its work on the States of California and Washington, the data pertaining to these two States are primarily chosen for analysis, although supplementary details are derived from the reports dealing with the outbreaks in other States of the Union. In following this plan, the commission does not intend to imply that botulism is primarily a problem of California or of the Pacific Coast States. The organization in the State of California enabled the commission to investigate details most carefully and trace sources of information, which were inaccessible in other States. It is not unlikely that many of the essential findings determined for the Pacific coast could be duplicated by the activities of a similar commission in other localities.

PART III.

HUMAN BOTULISM IN CALIFORNIA.

A. Single Cases or Those Occurring in Groups and Families, Proven by Bacteriological and Toxicological Tests.

PLANT PRODUCTS.

(a) STRING BEANS.

REPORT 1.—*String beans, purchased in open market and home canned.*

In January, 1916, at San Jose, Calif., an adult woman noticed an unpleasant odor when she opened a jar of beans. She tasted one pod and decided it was not spoiled, but when she heated the beans an offensive odor was given off. She then tasted another pod and decided that the beans were spoiled and discarded them.

Typical symptoms of botulism appeared within 24 hours and the patient died on the fifth day after tasting the beans. Necropsy showed marked congestion of the vessels of the meninges of the brain and cord and areas of broncho-pneumonia and hemorrhages in the lungs. On microscopic examination cellular thrombi were found in the blood vessels of various organs.

Laboratory tests showed a leukocytosis of 17,600 on the last day of life. The urine was normal.

Chickens which ate the discarded beans developed typical fowl botulism and died. *B. botulinus*, type A, was recovered from the crops.

Reported by Dickson. Monograph of Rockefeller Institute, No. 8, 1918, pp. 25–27.

REPORT 2.—*String beans, home grown and home canned.*

In January, 1917, at Escondido, Calif., eight persons ate salad prepared from uncooked canned string beans with mayonnaise. None noticed any unusual odor or taste when at table but one waitress, who had helped prepare the salad, stated that the contents of one of the two cans used did not taste quite right. Seven adults (five women and two men) developed typical symptoms of botulism and four died. Of the fatal cases, one developed symptoms within 24 hours, one on the second day, one on the fourth and one on the fifth day, while of those who recovered the onset of symptoms in two was on the second day and in one on the fourth day. Death occurred on the third, seventh, eighth, and twenty-sixth days.

Blood counts taken on the eleventh day in two cases which recovered and in one which died on the twenty-sixth day showed R. B. C. 5,300,000, 5,400,000, and 5,200,000, respectively, and W. B. C. 7,000, 13,000, and 12,500, respectively. The differential count was not unusual. The blood pressure in one case which recovered was 136/90 (Mercer).

Necropsy was performed in two cases. Neither showed broncho-pneumonia, but the vessels of one victim contained cellular thrombi.

B. botulinus was recovered from a can of beans of the same pack which remained in the cellar.

Reported by Dickson. Monograph of Rockefeller Institute, No. 8, 1918, pp. 30–37.

(*b*) Spinach.

Report 3—*Spinach, commercially canned.*

In October, 1920, at Oakland, Calif., a number of persons in a hospital consumed spinach which had been baked. Two No. 10 cans (about 78 ounces) had been opened and the contents placed in a pan and baked for about twenty minutes. When opened the spinach appeared good, but when heated an unpleasant odor was given off. A head nurse, who noted the odor, tasted a portion of the baked spinach from the top of the mass and told the chef to discard it as it was spoiled. The chef picked out what he believed to be spoiled, added the contents of another can, and served the new mixture. Those of the victims who were most severely poisoned ate several helpings of the spinach with vinegar, but at least one noted an unusual taste which she mentioned as tasting like cheese.

Six persons (three women and three men) developed typical symptoms of botulism, and two women and one man died. In four cases the onset of symptoms occurred between 24 and 36 hours after the ingestion of the poison, although one of the four patients was less severely poisoned and was not confined to her bed until the fifth day. She was the only one of the more serious cases to survive. The two remaining patients first showed symptoms on the third and fourth days after the meal, and were very mild cases. They both recovered. The nurse who tasted the cooked spinach from the top of the loaf did not become ill. Examinations of the blood of two patients who recovered showed R. B. C. 5,200,000 and 5,500,000, respectively, and W. B. C. 12,500 and 7,000, respectively. The differential counts showed nothing unusual.

Necropsy was performed in two cases. In both the bodies had been embalmed before the brains were removed and the degree of engorgement could not be determined. In one the blood vessels of the brain contained cellular thrombi.

One of the patients who died was given intravenous injections of a mixture of types A and B antitoxin, a few hours before she died, without any indication of even temporary benefit. Two patients who recovered were also given both types of antitoxin on the fifth and sixth days after ingestion of the poison, but there is no definite evidence that the course of the intoxication was influenced by it.

B. botulinus, type A, was isolated from the stools of two survivors on the sixth, seventh, and on the twelfth days, respectively, after ingestion of the poison. There was no material available for direct bacteriologic investigation.

Investigated by the commission. Reported by Geiger. U. S. Public Health Reports, 1920, vol. 35, p. 2858, and 1921, vol. 36, p. 1313.

Report 4.—*Spinach, commercially canned.*

In June, 1921, at San Diego, Calif., three persons ate spinach which had been boiled for 20 minutes in fresh water after removal from the can. On the following day one woman consumed the remaining portion of spinach after "heating it." There was nothing reported as unusual in the appearance, taste, or odor of the spinach even while being boiled.

Thirty-six hours after eating the "heated" spinach, the last woman developed typical symptoms of botulism, but those who ate the boiled spinach did not become ill. At the onset of symptoms, the patient took large doses of salts. She recovered.

The spinach was grown on farms fertilized with sewage in the same district where spinach which has been responsible for other outbreaks of botulism was grown. It was packed in Los Angeles in 1919, and had been in the grocer's stock for 18 months.

Washings from the can killed a guinea pig on the tenth day, and *B. botulinus*, type A, was isolated from the stool of the patient on the sixth day after she had eaten the spinach.

Investigated by the commission.

(*c*) APRICOTS.

REPORT 5.—*Apricots, home grown and home canned.*

In January, 1918, at Madera, Calif., eight persons (two men and two women, three boys, and one girl) developed typical symptoms of botulism after eating home canned apricots which had a peculiar taste and six died.

The onset of symptoms of seven patients occurred on the second day and of one on the third day. Two of the children died on the third day, the two women and one child died on the fourth day and the remaining child on the fifth day after ingesting the poison. The two men recovered after an illness lasting several weeks.

Necropsy performed on one child within a few hours after death showed marked engorgement of vessels of meninges, and a few small hemorrhages on the surface of the pons. There were patches of broncho-pneumonia in the lungs, and hyaline masses containing clumps of leukocytes in some of the vessels. Bacteriologic examination of the spleen and of the contents of the large and small intestines was negative for *B. botulinus.*

The two patients who recovered were given a mixture of types A and B immune goat serums by subcutaneous injection on the sixth, seventh, and eighth days of the intoxication.

The apricots were unsaleable windfall fruit and the jar containing them had been marked for early use because of fear of spoilage. No unusual odor was noted, but the fruit had a peculiar sharp taste.

Twenty-five chickens, one turkey, and a wild canary died after eating the discarded fruit. *B. botulinus*, type A, was recovered from the crop of one of the chickens.

Reported by Dickson. Arch. Int. Med., October, 1918, vol. 22, pp. 487–490.

(*d*) CORN.

REPORT 6.—*Corn, locally grown and home canned, cold-pack method.*

In January, 1917, at Corning, Calif., one adult female opened a jar of corn which appeared spoiled. Because of a cold she did not detect any unusual odor, but when she tasted the corn, she noted a biting or bitter taste.

Typical symptoms of botulism developed in 24 hours, but the patient recovered.

A number of chickens ate the discarded corn and developed typical fowl botulism. Many of them died. *B. botulinus* was recovered from the remnant of the corn but was not typed.

Records of the laboratory of the bureau of communicable diseases, California State Board of Health, Berkeley, Calif.

(*e*) OLIVES.

REPORT 7.—*Minced olives, commercially canned.*

In February, 1920, at Richmond, Calif., two persons, husband and wife, consumed minced olives in which they noticed nothing unusual in the taste or odor. On the day following the meal, the wife developed typical symptoms of botulism and died on the fifth day. The husband, who also ate some of the olives, but not as much as his wife, was not ill.

At necropsy, there was congestion of the viscera and a few petechial hemorrhages on serous surfaces. Characteristic thrombi were found in some of the vessels of the brain.

Antitoxin, types A and B, was given on the fourth day after the poisoning without beneficial results.

The minced olives were canned at Stockton in 1919. The empty can was recovered from the garbage can, and from washings of the can, botulinus toxin, type A, was demonstrated in three laboratories, and *B. botulinus*, type A, was isolated.

Investigated by the commission. Referred to by DeBord, Edmonson, and Thom. Jour. A. M. A., 1920, vol. 74, p. 1220.

B. Clinically Typical Cases, Single or in Groups and Families—Causative Food Assigned on Epidemiological Grounds Only.

(1) PLANT PRODUCTS.

(a) STRING BEANS.

REPORT 8.—*String beans, commercially canned.*

In May, 1910, at Oroville, Calif., four tramps robbed a grocery store, carrying away some canned string beans which were swells and definitely spoiled.

Typical symptoms of botulism developed and all the tramps died; two on the third day and two on the fifth day of illness. The hospital diagnosis at the time was cerebrospinal meningitis, but the clinical picture as shown by case records was typically botulism. No bacteriologic tests were made.

From case histories in the county hospital at Oroville, Calif.

Investigated by J. C. Geiger.

REPORT 9.—*String beans, locally grown and home canned.*

In Amador County, Calif., about 1912, six persons became ill and five died after eating home-canned string beans. The symptoms were those of botulism. Bacteriologic tests were not made.

Personal communication from Dr. La Forrest E. Phillips, Palo Alto, Calif. Reported by Dickson. Jour. A. M. A., 1917, *69*, p. 966.

REPORT 10. *String beans, home grown and home canned.*

In November, 1913, at Stanford University, California, 24 young people ate a meal at which bean salad was served. Two cans of beans had been used in preparing the salad, one commercially canned and one home canned, but their contents were easily distinguishable by their color. No unusual taste or odor was noted, but it was stated that the home canned beans were very tender.

Twelve students, 11 women and 1 man, became ill with symptoms typical of botulism, and 1 died. The onset of symptoms occurred as follows: 2 within 24 hours, 3 on the second day, 4 on the third day, and 4 on the fourth day after the meal. The patient who died showed symptoms on the second day and died on the fourteenth day after ingesting the poison. Of the 11 persons who recovered, 2 were only slightly ill and did not consult a physician, and 6 were treated by Dr. T. M. Williams, Palo Alto, Calif. Two patients were discharged from the hospital on the twenty-first day, 3 on the twenty-third day, and 1 on the fortieth day. All the survivors were apparently well in from four to eight weeks and all had returned to their university work within two months.

Necropsy was performed in the fatal case and showed hyperemia of the cerebral vessels and of the abdominal and thoracic viscera. The lungs showed areas of bronchopneumonia.

On microscopic examination Professor Ophüls observed peculiar cellular thrombi in the blood vessels of the meninges and brain, but no destruction of the finer structures of the ganglion cells was seen.

Anerobic cultures of the contents of the large and small intestines, cerebrospinal fluid, spleen, and heart's blood showed no growth of *B. botulinus.*

The beans were grown near Napa, Calif., and were canned at home, being processed by immersing the cans into boiling water for one hour on each of three consecutive days. None of the beans which were served were available for bacteriologic examination, but of two cans which were of the same lot, the contents of one were sour and contained viable spore-bearing bacilli which did not produce toxin.

Reported by Wilbur and Ophüls. Arch. Int. Med., 1914, vol. 14, p. 589. Additional notes by Dickson. Monograph of Rockefeller Institute, No. 8, 1918, p. 17.

REPORT 11.—*String beans, locally grown and home canned.*

In July, 1916, a woman at San Pasquale, Calif., noted that one jar of string beans which she had canned two weeks previously was leaking. She tasted one of the pods and noticed it was "slightly sharp," and when she began to "heat them over," that a strong disagreeable odor was given off. She then discarded the lot. Three days later she developed typical symptoms of botulism, but recovered after an illness of about two months. Seven months later she noticed that she became tired and hoarse after talking for some time.

A number of chickens which ate the discarded beans developed fowl botulism and about 25 died. There was no material for bacteriologic examination.

Reported by Dickson. Monograph of Rockefeller Institute, No. 8, 1918, p. 29.

REPORT 12.—*String beans, locally grown and home canned.*

In February, 1918, at Los Angeles, Calif., a woman noted an unusual taste in beans which she had canned during the previous summer and which she attributed at first to the presence of lemon juice which had been added at the time of canning. After tasting four pods, she concluded that the beans were spoiled and discarded them.

On the following day typical symptoms of botulism developed and she died on the third day after tasting the beans. Necropsy was not obtained.

A portion of the beans was recovered from the garbage can several days after they had been discarded, but bacteriologic examination failed to demonstrate *B. botulinus.*

Reported by Dickson. Arch. Int. Med., 1918, vol. 22, p. 491.

REPORT 13.—*String beans, home grown and home canned.*

In February, 1918, at Oakdale, Calif., a woman tasted one pod from a jar of string beans which had been given her by a neighbor who grew and canned them during the previous summer. She noted a peculiar irritating taste, but swallowed a portion. When she heated the beans, a very disagreeable odor was given off.

On the following day typical symptoms of botulism developed and the patient died on the fourth day after tasting the beans. Necropsy was not obtained.

The beans had been picked in the early morning within a few hours of the time they were canned. Twelve jars were prepared, and of these nine had been eaten after cooking without ill effect. In only two jars of the lot were the contents spoiled.

A portion of the discarded beans was recovered from the garden after they had lain in the rain for several days. *B. botulinus* was not isolated from them. The one remaining jar was also examined but neither toxin nor living bacteria were demonstrated.

Reported by Dickson. Arch. Int. Med., 1918, vol. 22, p. 490.

REPORT 14.—*String beans, locally grown and home canned.*

In May, 1918, near Fresno, Calif., a woman developed typical symptoms of botulism after eating home-canned string beans and died several days later.

Clinical and laboratory data are not available.

Personal communication from Dr. St. John Hely, Madera, Calif.

REPORT 15.—*String beans, home grown and home canned.*

In December, 1919, at San Rafael, Calif., a family of four (father, mother, boy aged 3, and girl aged 5) ate home-canned string beans direct from the jar as salad. There is no record that the beans were spoiled or that there was an unusual odor, but it is noted that they were not soft.

On the second day after the meal, typical symptoms of botulism developed in all four persons and the two children died on the fourth day, the mother on the fifth day, and the father on the seventh day after ingesting the poison.

A mixture of types A and B antitoxin was given to the father and mother without affecting the course of the intoxication. The mother received 50 cc. of each type intravenously and 50 cc. of each intramuscularly on the fourth day, but died on the fifth. The father received similar injections on the fourth day, 100 cc. of each intravenously on the fifth day, and 50 cc. of each intravenously on the sixth and seventh days, but he died on the evening of the seventh day.

Necropsy was performed in one case and cellular thrombi were demonstrated in the blood vessels of the brain. Cultures of the spleen and of the contents of the large and small intestines were negative for *B. botulinus.*

The beans had been grown at home and canned by the cold-pack method about three months before they were consumed. A portion of the beans had been cooked and served two weeks previously and had been consumed without ill effect. None of the beans were available for examination, but the contents of another jar which was obtained from the cellar did not contain *B. botulinus* or its toxin.

B. botulinus, type A, was recovered from the soil in which the beans had grown.

Investigated by the commission.

Report 16.—*String beans, home grown and home canned.*

In December, 1919, at Los Angeles, Calif., a woman tasted home-canned string beans which had spoiled. Thirty-six hours later she developed typical symptoms of botulism and died on the fifth day. Necropsy was not obtained.

The beans were grown at home and canned by the cold-pack method in September, 1919. None of the beans were available for laboratory tests.

Investigated by the commission.

Report 17.—*String beans, purchased in open market and home canned.*

In January, 1920, at Los Angeles, Calif., a woman opened a jar of string beans and tasted the contents. There was no apparent spoilage, and no unusual odor or taste was detected. She boiled the beans for from 35 to 50 minutes and served them to her three sons, who consumed them all.

On the following day the mother developed symptoms which were believed to be due to "bulbar paralysis," and she died on the fourth day after tasting the beans. The symptoms and course of her illness were typical of botulism. Necropsy was not obtained.

None of the three sons who ate the cooked beans developed any symptoms of illness.

The beans were purchased in the open market in October, 1919, and canned at home. No material was available for laboratory tests.

Investigated by the commission.

Report 18.—*String beans, locally grown and home canned.*

In April, 1920, at Los Angeles, Calif., a woman opened a jar of string beans which appeared spoiled, and she tasted a portion of two of the beans to verify the spoilage. On the following day she developed typical symptoms of botulism and died on the sixth day. Necropsy was not allowed.

The beans were grown in the vicinity and were canned at home in November, 1919.

A number of chickens which ate the discarded beans developed typical fowl botulism.

No material was available for laboratory tests.

Investigated by the commission.

(*b*) Olives.

Report 19.—*Ripe olives, commercially canned.*

In February, 1920, at Los Angeles, Calif., a woman ordered ripe olives with sandwiches in a department-store restaurant. She noticed that the olives had an unusually bitter taste. (She was familiar with the taste of ripe olives.) In about 12 hours she developed acute gastro-intestinal disturbances with nausea and vomiting, and these symptoms were followed by the typical symptoms of botulism. She was ill for about three weeks, but recovered.

The olives were canned in October, 1919, but none were available for bacteriologic examination, as they had been destroyed immediately after the customer became ill.

Investigated by the commission.

Report 20.—*Minced olives, commercially canned.*

In October, 1918, at Napa, Calif., a mother and her daughter aged 9 consumed sandwiches which had been prepared from minced olives. There is no recored of any indication of spoilage. On the following day the child developed typical sypmtoms of botulism and died 14 days after eating the food. The mother first noticed symptoms on the fifth day after ingesting the poison, but the course in her case was more rapid and she died on the seventh day.

Necropsy was performed in both cases and cellular thromb were observed in the blood vessels of the brain in one.

The minced olives were commercially canned and bore the label of a Long Beach (Calif.) packer. A portion of the paste which remained in the can was infused in water and the infusion was injected into guinea pigs. All of the animals which were injected died of typical symptoms of botulism, excepting those which had been injected with the boiled infusion. Tests for the isolation of *B. botulinus* were not completed.

Reported by the commission.

Report 21.—*Minced olives, commercially canned.*

In December, 1920, at Los Angeles, Calif. (New Year's eve), a number of persons consumed sandwiches prepared from canned minced olives. Nothing abnormal in taste or odor was detected. Five persons, four men and one woman, became ill with typical symptoms of botulism, but all recovered. The onset of symptoms in four cases occurred on the third day and in one on the fifth day.

The olives were canned at Stockton, Calif., in October, 1919, and were of the same lot which was responsible for outbreak No. 7. None of the material from the original can was available for bacteriologic examination, but from other cans of the same brand. *B. botulinus*, type A, was isolated, although no toxin could be demonstrated in saline infusion of the canned product.

Investigated by the commission.

(c) Apricots.

Report 22.—*Apricots, home canned.*

In March, 1915, at Fallbrook, Calif., five persons consumed home-canned apricots which were known to be spoiled, since there was a mold on the surface and the contents of the jar were disclored for about half their depth. The upper portion of the apricot was discarded and the remainder was served. It was noted that the apricots had a peculiar bitter taste, which was not displeasing to some members of the family.

All those who ate the apricots developed typical symptoms of botulism and all died. In three cases the onset of symptoms occurred on the third day, in one on the fourth, and in one on the fifth day. Two of the victims died on the fourth day, one on the fifth, one on the sixth, and one on the eleventh day after ingesting the poison. Necropsies were not obtained.

The apricots were canned at home.

A number of chickens which ate the discarded food developed typical fowl botulism and seven of them died. A dog also became paralyzed in the hind legs but recovered. Bacteriologic examination of the viscera and blood of one of the chickens was made in the coroner's laboratory, but no *B. botulinus* was found.

Reported by Dickson. Monograph of Rockefeller Institute, No. 8, 1918, p. 23.

Report 23.—*Apricots, locally grown and home canned.*

In January, 1918, at San Bernardino, Calif., two women consumed home-canned apricots which "did not taste just right." Both developed typical symptoms of botulism and both died.

There is no record of the exact time the poisonous food was eaten, but both victims developed symptoms on the same day and died on the third day after the onset. Necropsies were not obtained.

None of the poisonous food was available for bacteriologic examination, but other jars of the apricots from the cellar contained neither *B. botulinus* nor its toxin.

Reported by Dickson. Arch. Int. Med., 1918, vol. 22, p. 486.

(*d*) PEARS.

REPORT 24.—*Pears, locally grown and home canned.*

In January, 1910, at Sawtelle, Calif., 12 persons (3 men and 3 women, 2 boys and 4 girls) consumed home-canned pears which had been preserved four months previously. It was noticed that bubbles of gas escaped when the jar was opened and that the pears were "not exactly sour, but they tasted sharp."

All the members of the family developed typical symptoms of botulism and 11 died. In seven instances the onset of symptoms occured within 24 hours and in five on the second day after ingesting the poison. Three victims died on the second day, 6 on the third day, and 2 on the fourth day after the meal. The only person who recovered was the one who merely tasted a small portion of the pear when she opened the jar.

Necropsy was performed in one case, but no record is included in the report. Bacteriologic tests are not recorded.

Reported by Peck. Southern California Practitioner, 1910, vol. 25, p. 121.

REPORT 25.—*Pears, home canned.*

In March, 1918, at Colton, Calif., a man ate a portion of home-canned pears which had a peculiar taste. There is no record of other persons having eaten of the same lot. On the following day the patient developed gastrointestinal disturbances, which were followed by the typical symptoms of botulism. At the end of three weeks he was much improved, although the eye symptoms persisted, and he eventually recovered.

Material for laboratory tests was not available.

Reported by Dickson. Arch. Int. Med., 1918, vol. 22, p. 492

(*e*) ASPARAGUS.

REPORT 26.—*Asparagus, home canned.*

In November, 1915, a woman at Sacramento, Calif., opened a jar of home-canned asparagus and, although it was "a little sour," she drank the juice. When she heated the stalks a peculiar odor was given off, and the son discarded the cooked material because it tasted spoiled.

About six hours after drinking the juice the mother became nauseated and vomited and later developed the typical symptoms of botulism. She died on the fourth day after ingesting the poison.

Necropsy was not permitted, and no material was available for laboratory tests. The son who tasted the cooked asparagus did not become ill.

Reported by Dickson. Monograph of Rockefeller Institute, No. 8, 1918, p. 25.

(*f*) CORN.

REPORT 27.—*Corn, commercially canned.*

In April, 1918, at San Rafael, Calif., a woman ate three tablespoonfuls of canned corn which had a metallic taste, although it did not appear spoiled and had no unusual odor. The corn had been "warmed up," but not boiled. In about 11 hours she became ill. Two children, who also ate some of the corn, complained of stomach ache and some diarrhea, but showed no further symptoms after a dose of castor oil. The course of the woman's illness was typically botulism, but not severe. She recovered.

The corn was commercially canned, supposedly in Ohio. A portion of it was fed to chickens by the local health officer and four died of typical foul botulism. The contents of the crop of one was examined by Mrs. G. S. Burke, but no *B. botulinus* was found. No further laboratory tests are recorded.

Referred to by Burke, Jour. A. M. A., 1919, vol. 73, p. 1079, and investigated by the commission.

(*g*) Home-brew.

Report 28.—*Home-brew liquor.*

In September, 1919, near Colusa, Calif., six men imbibed freely of home-brew, which had been prepared from various home-canned products. One of the men could not be located after the party. Two of the men had been drinking the liquor at frequent intervals for two days before the party and they both became ill on the day following, but the other three did not develop any symptoms until the third day after ingesting the poison. Two of the victims died on the seventh day and two on the ninth. The patient who recovered possibly owes his life to the fact that his wife gave him a very large dose of physic when he returned home.

Necropsies were not obtained.

The liquor was prepared by placing canned peaches, apricots, plums, grapes, and tomatoes in a vinegar barrel which they filled with water. The mixture was allowed to ferment for 10 days. The fruit had been canned at home four and five years previously. Analysis showed that the liquor contained from 11 to 15 per cent alcohol.

The laboratory tests were not conclusive, but little material was available for examination as the barrel of liquor had been emptied. A number of hogs on the ranch developed paralysis, suggesting botulism, and died presumably after having consumed the contents of the barrel.

B. botulinus was isolated from the soil in the garden where the fruit had been grown.

Reported by Kelley, California State Board of Health bulletin, October, 1919, vol. 15, p. 114.

Investigated by the commission.

(2) ANIMAL PRODUCTS.

(*a*) Beef.

Report 29.—*Tamale (beef) source unknown.*

In May, 1899, at Los Angeles, Calif., a woman became ill with symptoms resembling botulism after eating a beef tamale which appeared to be good. The onset of symptoms occurred within 24 hours of the meal. During the course of the illness there were features which suggested intercurrent infection, but the patient eventually recovered.

No laboratory tests are recorded.

Reported by Lewis. Southern California Practitioner, 1899, vol. 14, p. 464.

Report 30.—*Beef.*

In November, 1902, at San Francisco, Calif., seven persons (three men, two women, and two whose sex is not stated) became ill in a boarding house after eating "tainted boiled beef." Three of the victims died. The recorded onset of symptoms occurred as follows: One within 24 hours, two on the second day, one on the third day, one on the fourth day, and one on the sixth day after ingesting the poison and the course of the illness was typical of botulinus intoxication. The three deaths occurred on the fourth, fourteenth, and sixteenth days.

Necropsy was performed in one case and engorgement of the capillaries of the medulla, pons and cerebellum and patches of broncho-pneumonia are recorded. There is no record of microscopic examination.

Reported by Jellineck. Pacific Medical Journal, 1903, vol. 46, p. 110, and California State Journal of Medicine, 1902–3, vol. 1, p. 121.

Report 31.—*Calf's-head vinegarette, home prepared.*

About eight years ago, at Los Angeles, Calif., a man developed typical symptoms of botulism and died. He believed his illness was caused by eating home-prepared calf's-head vinegarette. No laboratory data are available.

Reported to the commission.

(b) Pork and Beans.

Report 32.—*Pork and beans, commercially canned.*

In December, 1906, three persons (two men and one woman) developed typical symptoms of botulism after eating commercially canned pork and beans. It is stated that there was nothing abnormal in the appearance, taste, or odor of the beans. The onset of symptoms occurred within 24 hours in two cases and on the second day in one. One of the victims died on the fourth day and two on the fifth day after eating the beans.

A number of chickens were fed with the remnants of the beans and all developed typical fowl botulism; nine of them died.

Laboratory tests are not recorded.

Reported by Sheppard. Southern California Practitioner, 1907, vol. 22, p. 370.

(c) Pork.

Report 33.—*Ham, home cured.*

In October, 1917, at Los Angeles, Calif., two persons developed typical symptoms of botulism and died after eating home-cured ham. The onset of symptoms occurred within 24 hours and death occurred on the fifth and sixth days after ingesting the poison.

There is no record of laboratory investigation.

Reported to the commission.

Report 34.—*Sausage, commercially prepared.*

In January, 1915, at Los Angeles, Calif., two persons, man and wife, developed typical symptoms of botulism which were attributed to toxin in wiener-wurst. The onset of symptoms occurred within 24 hours and death on the eighth and thirteenth days after ingesting the poison.

Necropsy was performed in one case and congestion of the viscera noted.

There is no record of laboratory investigation.

Reported by Frost. American Medicine, 1915, N. S. 10, p. 85.

(d) Clams and Fish.

Report 35.—*Clam juice, commercially bottled.*

In October, 1913, at San Francisco, Calif., three men drank clam juice which was prepared in a saloon by adding hot water to bottled clam broth. Two of them developed typical symptoms of botulism and died on the third and fifteenth days after ingesting the poison. The third man showed no symptoms excepting a slight gastro-intestinal disturbance.

It is recorded that the bartender drank from 20 to 30 drinks of mixed variety daily and that he had imbibed as much alcohol as usual on the day he drank the poisonous broth.

Necropsies were not obtained and no material was available for laboratory tests.

Reported by Bine. Boston Medical and Surgical Journal, 1917, pp. 177–559.

Report 36.—*Clam broth, commercially bottled.*

In 1912, at San Jose, Calif., two men developed typical symptoms of botulism after drinking clam juice in a barroom. One died.

No laboratory tests were made and no further data were available.

Reported to Dickson by attending physician in 1916. Jour. A. M. A., 1917, vol. 69, p. 966.

Report 37.—*Tuna, commercially canned.*

In February, 1918, at Los Angeles, Calif., a man tasted a portion of canned tuna which he found to be spoiled and consequently discarded it. About three days later he developed typical symptoms of botulism and died on the sixth day after tasting the tuna.

There is no record of necropsy and no material was available for laboratory examination.

On the day after the tuna was discarded, fourteen chickens died after an illness typical of fowl botulism.

Reported to the commission in 1921 by the attending physicians.

Report 38.—*Probably fish pickled in vinegar, home prepared.*

On July 7, 1921, at San Jacinto, Calif., a woman opened a jar of home-canned white beans which she tasted and discarded because they were spoiled. The beans were fed to a number of chickens. On the following day she opened one or more jars of home-canned pickled mackerel and herring which had a spoiled appearance and odor, notwithstanding which she tasted the contents of the jar before throwing them to the chickens. Two days later, July 10, the woman developed typical symptoms of botulism and died seven days after tasting the fish. Necropsy was not obtained.

On July 9, the day following that upon which the pickled fish was discarded, and two days after the beans were discarded, a number of chickens showed typical symptoms of botulism and 75 died within three days. A cat which is known to have eaten the fish, but presumably ate none of the beans, developed typical symptoms of botulism but recovered.

The fish had been caught on June 25 or 26, less than two weeks before the food was tasted and were prepared as follows: The fish were eviscerated, cleaned, washed in fresh and salt water, and then sprinkled with salt. They were then washed and cut into small pieces and packed into Mason glass pint and quart jars which had been boiled for 30 minutes in weak soda solution. There was no indication of spoilage at this time. In the jars the fish was covered with vinegar and salt and to some of them tomatoes were added. The covers were applied and the jars were placed in a cooker and cooked for five hours, presumably at a boiling point although there is some doubt as to whether that temperature was maintained throughout.

A number of chickens were sent to the laboratory for examination. *B. botulinus*, type A, was recovered from the contents of the crops, gizzards, and intestines of two chickens and botulinus toxin, type A, was demonstrated in the contents of the crops.

Investigated by the California State Board of Health, the Bureau of Chemistry, and the commission.

(*e*) Cheese.

Report 39.—*Cottage cheese, home prepared.*

In June, 1912, at Long Beach, Calif., seven persons developed typical symptoms of botulism and two died after eating home-prepared cottage cheese.

No laboratory data are available.

Reported to the commission by the attending physician in 1921.

C. CASES OF BOTULISM—CAUSATIVE FOOD UNKNOWN.

(*a*) anatomically or bacteriologically proven.

Report 40.—*Causative food unknown.*

In May, 1916, at San Francisco, Calif., a man developed typical symptoms of botulism and died on the fourth day after the onset of his illness.

The cause of the poisoning was not determined. Laboratory examinations showed leucocyte counts of 10,000 and 14,000. The cerebrospinal fluid contained 80 cells but the type was not stated in the report. The urine was clear until the third day of illness when albumin, hyaline casts, and red blood cells appeared.

Partial necropsy was performed and extreme congestion of the meninges and substance of the brain was noted. On microscopic examination cellular thrombi were observed in some of the blood vessels of the meninges.

Reported by Dickson. Monograph of the Rockefeller Institute, No. 8, 1918, p. 27.

Report 41.—*Causative food unknown.*

In April, 1921, near San Luis Obispo, Calif., an ex-service man became ill and was sent to the marine hospital at San Francisco. He died several days later. A diagnosis of epidemic encephalitis was made, but from a study of the records after the patient's death the symptomatology and course more closely resembled botulism.

Necropsy was performed and congestion of the brain and meninges and the viscera was noted. On microscopic examination, a few vessels were seen in the brain in which a slight perivascular infiltration was present. No cellular thrombi were found. On bacteriologic examination of the brain tissue, *B. botulinus*, type B, was recovered.

Reported by Geiger. U. S. Public Health Reports, 1921, vol. 36, p. 1663.

(*b*) CLINICALLY TYPICAL CASES.

Report 42.—*Causative food unknown.*

In 1913, at Hornbrook, Calif., a woman developed symptoms of botulism and died. No cause for the poisoning could be determined, but it was noted that she had been caring for a number of sick chickens just before her illness.

Necropsy was not performed.

Reported by Dickson. Jour. A. M. A., 1917, vol. 69, p. 966.

Report 43.—*Causative food unknown.*

In March, 1911, at Los Angeles, Calif., a man developed typical symptoms of botulism and died seven days after the beginning of the illness.

Necropsy is not recorded.

Reported by Frost, Amer. Med., 1915, N. S. 10, p. 85.

Report 44.—*Causative food unknown.*

In November, 1916, at Berkeley, Calif., three men students at the University of California developed typical symptoms of botulism but all recovered. No material for laboratory tests was available.

Reported by attending physician to the commission.

Report 45.—*Causative food unknown.*

In September, 1920, a family who were motoring through were obliged to stop at Corning, Calif., because of the illness of their daughter, aged 9. The symptoms were typical of botulism and the child was very ill, but recovered. There was no record of the child having eaten spoiled food and no other member of the family was ill.

Reported to the California State Board of Health and investigated by the commission.

Since 1889, a total of 45 instances of human botulism have been reported for the State of California. A total of 132 people, with 81 deaths, are involved in these observations. According to Table 2, 42 of the single or groups of cases occurred since 1910; in fact the majority of the outbreaks were recognized during the last five years.

TABLE 2.

Year.	Plant products.				Animal products.				Un-known.
	Home preserved.		Commercially preserved.		Home preserved.		Commercially preserved.		
	Bacteriologically proven.	Bacteriologically not proven.	Bacteriologically proven.	Bacteriologically not proven.	Bacteriologically proven.	Bacteriologically not proven.	Bacteriologically proven.	Bacteriologically not proven.	
1899–1909	0	0	0	0	0	1	0	2	0
1910	0	1	0	1	0	0	0	0	0
1911	0	0	0	0	0	0	0	0	0
1912	0	1	0	0	0	1	0	1	0
1013	0	1	0	0	0	0	0	1	1
1914	0	0	0	0	0	0	0	0	0
1915	0	2	0	1	0	0	0	1	1
1916	1	1	0	0	0	0	0	0	1
1917	2	0	0	0	0	1	0	2	0
1918	1	5	0	1	0	0	0	0	0
1919	0	3	0	0	0	0	0	0	1
1920	0	2	2	1	0	0	0	0	1
1921 (until June 30)	0	0	1	1	0	1	0	0	1
Total, 45	4	16	3	5	0	4	0	7	6
Bacteriologically and toxicologically proven	4		3		0		0		0
Not proven and inconclusive.	16		5						
Total, 45	20		8		4		7		6

It is significant that nearly one half of the reports deal with single, isolated cases, while the remaining 20 observations involve groups and families. The following explanation can be offered for this interesting fact: In eight of the records of single cases it is stated that the patient tasted the preserved food in order to confirm certain apparent signs of spoilage. In the majority of instances, the food, which was found unfit for consumption, was then discarded. Its poisonous character was subsequently proved either by the illness or death of the housewife or by the occurrence of botulism among the chickens, which had access to it. In two instances (reports 17 and 26) the spoiled food was boiled and later consumed by other members of the household without consequences.

AGE AND SEX DISTRIBUTION.

There is no evidence of any special predisposition at any age period. The age and the sex incidence in any outbreak seems to depend entirely upon the accidental distribution of those who ate the food. It is of interest to note that according to the single case reports more females were poisoned than males. This observation is readily explained by the fact mentioned above that the housewives tasted or sampled the spoiled food. The sex distribution for 115 cases can be summarized in the following formula:

Children: Males: Females = 1:3.13:3.46.

INTOXICATION RATE.

As a rule all who ate the poisonous food became ill. The recorded clinical cases in individual outbreaks often form 100 per cent of those consuming the food. In only two instances (reports 2 and 6) is it recorded that one person in each outbreak failed to develop symptoms after eating the spoiled vegetables or fruits. It seems reasonable to presume that the person in report 2 who escaped illness received beans only from the unspoiled jar. The case mentioned in report 6 is not readily explained. Either the toxin of *B. botulinus* was unevenly distributed in the rather dry compact mass of minced olives or the husband who claims to have eaten the same amount of relish as his wife possessed some resistance toward the poison. An uneven distribution of the toxin in the food is probably exceptionally rare, but Römer (5) reported the fact that several people who ate of the portion of a ham that had a normal appearance remained well, while those who consumed meat from the deep green-grayish discolored parts developed typical symptoms. These observations should be taken into consideration when the intoxication percentage is being estimated.

In comparison with the usual food infection and poisoning outbreaks, the botulism intoxication rate appears rather high. In outbreaks due to food intoxications and food poisoning 50 per cent and in some instances even 80 per cent or more of those consuming the food become ill; but inasmuch as very few careful epidemiological studies of food poisoning have been made, it is not unlikely that in these instances individuals or groups are overlooked who consumed the food without harm. The available data, therefore, do not permit of a comparison of the infectivity rate of food infections with the intoxication rate of botulism.

CASE MORTALITY RATE.

The number of fatal cases varies greatly in different outbreaks. In 20 group and family reports involving from 2 to 12 persons per outbreak, the mortality varied from 0 to 100 per cent. As a rule, more than 50 per cent succumbed to the intoxication. In 45 outbreaks, there were 132 cases with 81 deaths, or a case mortality rate of 61.3 per cent. For 39 outbreaks in which the causative agent was known, the mortality was likewise 62.4 per cent. For 3 outbreaks (reports 2, 3, and 5) definitely shown to be due to *B. botulinus* toxin, the case mortality rate was 61.9 per cent. It is not unlikely that the toxicity and the amount of the causative food consumed or better the actual quantitative intake of the poison influences the mortality rate, although there are probably several factors which control the rate of absorption and thus cause individual variations in the rapidity of onset and severity of symptoms. Concomitant ingestion of other microorganisms in the spoiled toxic food may also influence the death rate. In children the mortality rate is higher than in adults. It is not unlikely that a similar quantitative relationship between body weight, toxin intake and mortality exists, as has been observed in guinea pigs and mice.

According to Savage (6) the mortality in the British food-poisoning outbreaks does not exceed 1.5 per cent. Likewise in Germany, the mortality rate was for the years 1868–1898, according to Schneidemuhl (7) approximately 1.52 per cent. It is therefore obvious that botulism, in contrast to other forms of food poisoning, is characterized by an exceptionally high case mortality rate.

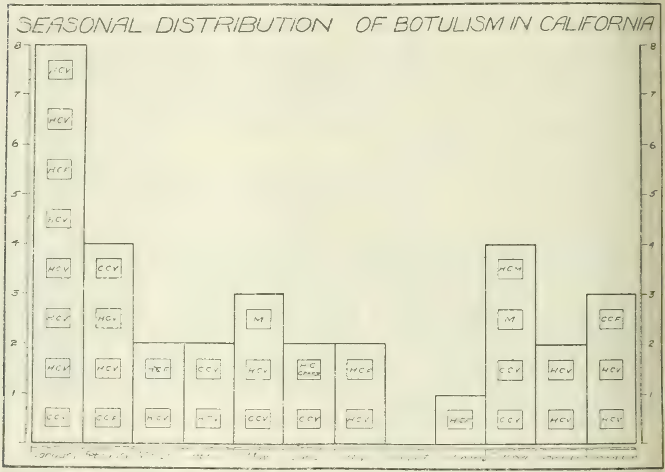


FIG. 2. H. C. V., home canned vegetables; H. C. F., home canned fruits; C. C. V., commercially canned vegetables; C. C. F., commercially canned olives or fish; M., meat; H. C. M., home prepared meats.

SEASONAL DISTRIBUTION.

In California, more than one-half of all the botulism outbreaks have occurred during the fall and winter months, namely October–February. This fact is well illustrated in figure 2. In contrast to the bacterial food infections, which are usually prevalent during the hotter months, botulism bears no relation to the climate, because it is intimately connected with the consumption of preserved vegetables, string beans, corn, spinach and asparagus, pickled olives, and occasionally home-canned fruits (apricots and pears) which takes place during the winter season. How far the temperature of the subtropical summer climate of California influences the occurrence of botulism will be considered in connection with the discussion of the foodstuffs responsible for this intoxication.

FOODSTUFFS RESPONSIBLE FOR THE OUTBREAKS.

It has been pointed out repeatedly by Dickson that in the United States the majority of botulism cases are caused by the ingestion of canned vegetables or fruits in which the organism had grown and produced its toxin. This statement is borne out by the graphic presentation of the causative foods in figure 3. According to the reports in which the responsible food has been presumed to be the causative agent or has been toxicologically or bacteriologically proven, so 62.3 per cent of the outbreaks in California were due to home canned string beans, asparagus, corn, commercially packed ripe olives and spinach and home canned fruits (apricots and pears)· while only 24.4 per cent were produced by animal products.

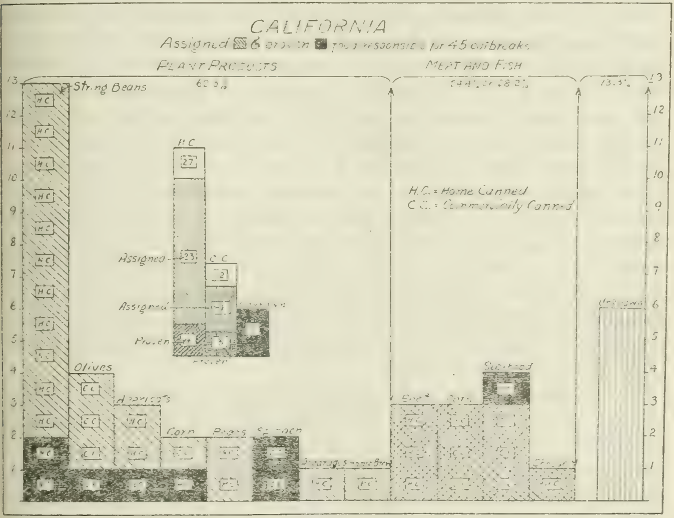


FIG. 3.

String beans played a very important rôle, a little less than one-half of all the outbreaks due to vegetable products followed the tasting or consumption of this food in a preserved state. Experimental studies have shown that string beans furnish an excellent media for the elaboration of a very potent toxin, and that spoilage of this vegetable when preserved is sometimes so slight that the housewife or cook will fail to detect it. Furthermore, canned string beans are frequently served cold as salads and any odor, which might arouse suspicion, is usually masked by the use of vinegar or mayonnaise dressing. Probably similar difficulties of detecting slight spoilage are responsible for the outbreaks due to minced olives. This relish is highly spiced and rather dry; it is consumed cold and volatilization of the fatty acids which would indicate spoilage is exceedingly slight in the first few hours following the opening of the can. Numerous laboratory examinations justify this statement.

It has been proven that *B. botulinus* can grow unaided in olives, spinach, corn, asparagus, beets, peas, artichokes, apricots, peaches, pears, prunes, and so forth, under certain conditions and cases of poisoning due to almost all of these products have been reported. However, it is exceedingly difficult to grow *B. botulinus* in the majority of fruits and some vegetables, if they are fresh and sound at the time of canning. In report 5, it is stated that the apricots were unsalable and windfall, "moldy" (report 22) or "obviously spoiled" (reports 23 and 24). Some observations also suggest that fermentation and toxin production due to *B. botulinus* occurs with much greater facility in the presence of concomitant anaerobes or even aerobes and fungi. Little need be said concerning the possible highly toxic properties of the so-called home-brew liquors. Fermenting mixtures of fruits and vegetables, provided a sufficient amount of water has been added, may favor the growth of *B. botulinus* and the formation of its toxin. It is therefore not unlikely that, as in report 28, some of the fatalities which have followed the drinking of undistilled home-brew liquors may have been due to the poison of *B. botulinus*. Toxicologists and prohibition officers should keep this possibility in mind.

On account of the high protein content, the suitable reaction and the degree of moisture, meat, fish and milk products can serve excellently as mediums for the development of *B. botulinus*. It is therefore rather surprising that in this series the number of cases due to these products is comparatively small. Unfortunately neither of these cases has been proven bacteriologically or toxicologically. In several instances (for example report 38) vegetable products were consumed at the same time and it is not unlikely that the food held responsible for the outbreak was not at fault. Furthermore, it is impossible to determine from the available records what contributory factors may lead to the elaboration of the *B. botulinus* toxin. In a few instances (reports 32, 36, 37, and 38) the products were canned while in others (reports 33, 34, 35, and 39) various other methods of preservation produced the condition suitable for the formation of the specific toxin. In every instance, regardless of the method of preservation, the foods were consumed either in a raw or imperfectly cooked state. From the data presented, it is evident that animal food products play a subordinate rôle in the botulism outbreaks of this country. Notwithstanding this, attempts have repeatedly been made to compare the local conditions with those in Europe.

It is not entirely clear why in European countries pork or meat products are the most frequent causes of botulism whereas in the United States preserved plant products are usually responsible, although it is a fact that in those portions of Europe where botulism is most frequent, there is a habitual consumption of uncooked, preserved meats and that in America canned vegetables and fruits, which have not been cooked after the removal from the container, are widely used as salad. It can therefore be stated that in all probability the differences in the tastes and dietaries of European and American people are responsible for the differences in incidence.

The case mortality rate varies somewhat according to the foodstuff involved in the outbreak.

Table 3.

Mortality from—	Number of outbreaks.	Number of cases.	Number of deaths.	Percentage.
Home canned string beans	5	33	18	54.4
Commercially canned spinach	2	7	3	42.8
Commercially pickled and bottled olives	4	9	3	33.3
Home-canned apricots	3	15	13	86.6
Home and commercially canned meat, fish, and milk	7	26	15	57.6

It is difficult to determine from the histories of the outbreaks the contributory factors which favor the germination of the spores of the toxicogenic bacillus. In another paper it will be definitely shown that *B. botulinus* is an organism of the soil. Spores of the organism are sometimes quite abundant, and any food product contaminated with soil may occasionally carry these potential elements. Moreover, it is well known from the work of Dickson and Burke, Weiss, and others that the spores of the majority of recently isolated strains are very resistant to heat. They share this property with other representatives of the group of proteolytic anaerobes. Any method employed in the preservation of food which fails to consider these facts is apt to favor spoilage, and consequently, in a certain small percentage of instances, the growth of *B. botulinus* and its toxin. It may therefore be stated without fear of contradiction that in the majority of outbreaks underprocessed, and therefore improperly preserved, nonsterile plant products have been the vehicles for the toxin. These conclusions apply to the commercially as well as to the home canned products.

Commercially pickled and bottled olives or factory-canned vegetables (spinach) were responsible for 6 outbreaks, while home-canned products were the causative food in 20 instances. The cold-pack method was frequently employed for the canning of vegetables and fruits at home, and in this series apparently was responsible for the death of 18 people. While there is no necessity of entering into a controversy relative to the economic merits of the two methods of food preservation, the epidemiologic observations have established the fact that even the commercial packing methods have been in few instances inadequate to insure freedom from botulinus poison. It is not surprising that the various home-canning procedures are inefficient when one realizes that the commercial canning industry, which for years has labored with improved machinery, a trained personnel, and in many instances with scientifically controlled processes has not entirely succeeded in eliminating spoilage and with it the occasional occurrence of botulism. From a public-health standpoint it is essential that all canning methods, whether commercial or at home, should aim at absolute sterility of the product to insure absolute freedom from *B. botulinus* and its toxin. In the light of recent studies this prerequisite does not offer unsurmountable difficulties, provided fresh and sound vegetables and fruits are used for preservation.

No information can be gained from these reports relative to the influence of salts, sugar, or organic acids. In one instance (report 12) it is stated that lemon juice had been added to the jar of string beans, yet the liquor was toxic and caused a fatal case of botulism.

The reports were also analyzed in order to detect any relationship between the toxicity and the time which elapsed from the act of canning and the tasting or eating of the preserved food. In the majority of instances the products had been preserved for from 2 to 26 months previous to consumption. In two cases (reports 11 and 38), however, it is stated that the canned string beans or fish were only two weeks old. Unquestionably the summer heat of July assisted in the production of the toxin. These facts also indicate that in some instances at least the germination of the spores, which had been subjected to heat during processing, was not retarded to any noteworthy degree. The commission is in possession of an epidemiological observation (see report 17 on botulism in chickens) which suggests that even in a period of three days sufficient toxin may be produced in a half-gallon jar of string beans, canned by the cold-pack method, to be fatal when fed to chickens. There is no evidence that food originally toxic may become nontoxic in the course of storage for several months, although in the light of recent laboratory studies such conditions might be expected. Future investigations dealing with the examination of spoiled food should carefully consider these facts, and such material should not only be tested toxicologically but also bacteriologically for the presence of *B. botulinus* spores. At times certain cans of spinach and olives belonging to the same lot, which was responsible for cases of botulism contained viable toxin-producing organisms, although no toxin was present. As the housewives or cooks who conducted the canning are usually among the first victims to die, many of the contributory factors which assisted in the elaboration of toxins in the preserved food could unfortunately not be ascertained.

PHYSICAL APPEARANCE OF THE INCRIMINATED FOOD.

The prevalent idea that the food must always be visibly spoiled in order to be toxic is not entirely supported by the available observations, although there is no doubt that in the majority of instances the canned product is so spoiled as to readily attract the attention of the one who opens the container. In order to emphasize this very important point, it is deemed advisable to tabulate the various statements as they appear in the history of the outbreaks.

TABLE 4.—*Home-canned vegetables and fruits.*

Report 1.—String beans: "Odor unpleasant."
Report 2.—String beans: "Taste not right."
Reports 9, 11.—String beans: Jar leaking. "Sharp biting taste; on cooking, disagreeable odor.'
Report 12.—String beans: Peculiar taste attributed to lemon juice.
Report 13.—String beans: On boiling, disagreeable odor; peculiar irritating taste.
Report 15.—String beans: Beans hard and supposedly not spoiled.
Report 17.—String beans: No evident spoilage, no bad odor or taste.
Report 5.—Apricots: No odor, but "sharp taste."
Report 22.—Apricots: Top of jar moldy, lower portion tasting bitter.
Report 23.—Apricots: "Did not taste just right."
Report 6.—Corn: Spoiled, but odor not detected on account of cold. Taste bitter or biting.
Report 27.—Corn: No spoilage, no odor, but taste "metallic."
Report 24.—Pears: Obviously spoiled and taste not right.
Report 26.—Asparagus: A "little sour," on heating, offensive odor.

TABLE 5.—*Commercially canned vegetables and fruits.*

Report 3.—Spinach: Visible spoilage and repulsive odor on heating.
Report 4.—Spinach: Neither spoiled in appearance or taste or odor on boiling.
Report 7.—Minced olives: No odor or bad taste, but can emanated definite odor.
Report 19.—Minced olives: "Bitter or of unusual taste."
Report 21.—Minced olives: "No evident signs of spoilage."

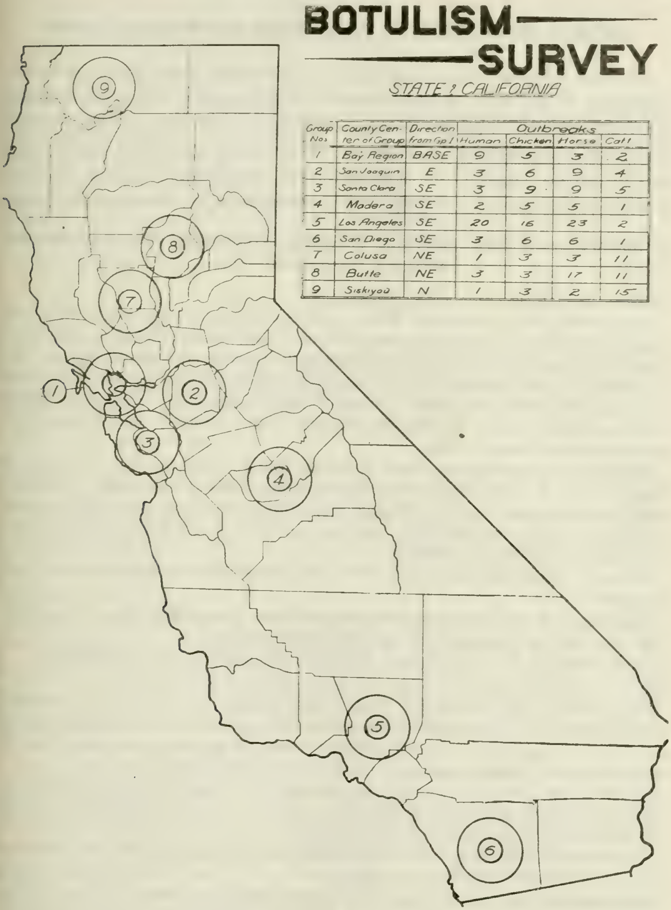
From the above tabulation (Tables 4 and 5) it must be concluded that in about one-half of the instances the spoilage of the preserved product was so striking as to attract the attention of the person opening the can, yet in some instances the disintegration of the contents was so slight that the housewife was prompted to confirm her impression by tasting it. As a rule she found the taste of the poisonous food to be abnormal or peculiar. In some cases the spoilage was not noticed until the food became heated during the process of cooking.

According to reports 15, 17, 4, and 21, macroscopic spoilage in the form of abnormal consistency or appearance was certainly not visible. These facts are supported by repeated observations made in several laboratories by independent workers. Several jars or cans of string beans, beets, asparagus, olives and so forth, may appear perfectly normal according to the verdict of a large number of persons familiar with the peculiar odor of anaerobic vegetable or fruit fermentation, although they contain a highly potent toxin. Food products containing a large amount of protein or starch, as peas corn, fish and meat, are apt to show marked physical disintegration and other signs of spoilage in the form of leaking jars or bulging cans. It will be shown later that such food is usually considered unfit for human consumption, but it has in many cases been fed with fatal results to chickens. From the observations recorded in reports 15, 17, 4, and 21, it becomes evident that it is unwise to place absolute reliance on the dogmatic assertion that only food which is obviously unfit or putrid is liable to contain the toxin of *B. botulinus.*

In this connection, attention is called to the interesting observation recorded in report 4. Spinach, neither spoiled in appearance, taste, or odor, had been boiled for 20 minutes in fresh water and had been consumed by three people without any bad effect. The remainder of the vegetable was put aside, heated the next day and consumed by a woman who developed a mild attack of botulism. It is most likely that the interior portion of the boiled spinach still contained some poison, but it is also possible that during the lapse of a day in the warm climate sufficient toxin developed from the large number of spores which were originally in the under processed can and which survived the boiling. This observation is described in detail in order to emphasize how difficult the prevention of human botulism will be as long as unsterile, underprocessed canned products, which may contain viable spores of *B. botulinus* are being used by the public as food, although it must be stated that not all preserved foods can be processed until they are sterile, because the temperatures necessary to destroy the spores of thermophilic bacteria would render the food unmarketable. Preservation of any vegetable, fruit or animal product can only be regarded as safe, however, when spores of *B. botulinus* can not survive the processes. When once this prerequisite has been attained, the uncertainty and uneasiness which is felt by the consumer in judging or in deciding the ultimate disposal of an apparently sound or slightly spoiled can or jar of preserved food, will be completely eliminated.

GEOGRAPHICAL DISTRIBUTION.

A study of Figure 4 and its legend indicates that the outbreaks occur in all parts of the State, but that they are more frequent in the southern section. This localization is closely associated with the distribution of *B. botulinus* spores in nature, particularly in the soil. The relationship between prevalence of *B. botulinus* in the soil and the frequency of botulism is also illustrated by the fact that 23 outbreaks due to home-canned vegetables or fruits occurred in rural communities. Furthermore, the majority of the rural outbreaks were caused by home-canned products, while in the 6 urban outbreaks, as could be expected, commercially pickled olives and canned spinach were responsible. Most of the outbreaks due to animal or fish products occurred in cities. It is not unlikely that in all cases the causative food originated in the rural districts. The interesting observation that there may be a geographical localization of botulism in certain sections of a city or village (reports 16, 17, and 18) is partly corroborated by the recently proven prevalence of *B. botulinus* spores in the soil of these areas.



Group Nos	County Center of Group	Direction from Gp I	Outbreaks			
			Human	Chicken	Horse	Catl
1	Bay Region	BASE	9	5	3	2
2	San Joaquin	E	3	6	9	4
3	Santa Clara	SE	3	9	9	5
4	Madera	SE	2	5	5	1
5	Los Angeles	SE	20	16	23	2
6	San Diego	SE	3	6	6	1
7	Colusa	NE	1	3	3	11
8	Butte	NE	3	3	17	11
9	Siskiyou	N	1	3	2	15

FIG. 4

PART IV.

HUMAN BOTULISM IN THE STATE OF WASHINGTON.

Several reports which appeared in the daily press indicated that botulism is not rare in the State of Washington. The commission has collected the reports dealing with two outbreaks in Seattle and with one case observed in Monroe. During the month of April, 1921, an intensive survey on botulism was conducted in the Yakima Valley. The report covering this investigation is presented in detail.

A. Single Cases or Those Occurring in Groups and Families, Proven by Bacteriological and Toxicological Tests.

REPORT 46.—*String beans, home canned.*

In February, 1921, at Seattle, Wash., a woman opened a jar of beans which was "gassy and foamy," had a pungent odor and a sour taste. Notwithstanding the apparent spoilage, she ate five or six small pieces and discarded the remainder. She developed typical symptoms of botulism within twenty-four hours and died on the third day after eating the beans. Necropsy was not performed. A maid also tasted one small piece of bean, but immediately spat it out because of the offensive taste. She did not suffer any ill effect.

The beans had been grown upon a farm near Seattle where one year before several hogs had been ill with symptoms resembling botulism and two had died. The beans were packed at home by the cold-pack method, and the victim stated that she had followed all directions explicitly. Spoilage was noted in a number of jars of beans and other home-canned products.

A rooster which had eaten some of the discarded beans developed typical fowl botulism.

A portion of the discarded beans was recovered from the garbage can. *B. botulinus*, type A, toxin was demonstrated and the bacillus isolated.

Investigated by Dr. C. R. Fellers, Seattle, at the request of the commission.

REPORT 47.—*Asparagus, purchased in open market and home canned.*

In November, 1917, at Seattle, Wash., three persons (two men and one woman) developed typical symptoms of botulism after eating home-canned asparagus, and all died. The symptoms in one case occurred within 24 hours; in the others there is no exact record. Death occurred on the fourth day after eating the salad.

Necropsy was performed in two cases, but after the bodies had been embalmed. There was marked congestion of the viscera and microscopic examination showed cellular thrombi in the vessels of both cases.

A number of chickens died after eating the discarded asparagus, and the contents of the crops contained *B. botulinus*, type A.

The asparagus had been purchased in the open market and canned at home by the method described in the pamphlet issued by the manufacturer of the glass jars which were used. When the first jar was opened, the asparagus was cooked but did not taste right, and the housewife told the maid to discard the lot. Instead of doing so the maid gave the remainder of the pack to a friend, at whose home the poisoning occurred.

When the second jar was opened, half the contents was cooked and eaten. None who ate the cooked asparagus became ill. The remainder was served after "warming up" on the following day to one man, who was the first victim of the poisoning. Four days later a third jar was opened and the contents served as salad to two persons, both of whom succumbed to botulinus intoxication.

The woman who opened the asparagus did not note anything unusual, and only one of the three victims detected any unusual odor.

Reported by Dickson. Arch. of Int. Med., 1918, *22*, p. 485.

REPORT 48.—*Spinach, locally grown and home canned.*

In May, 1919, at McKenna, Wash., three persons (father, mother, and adult son) developed typical symptoms of botulism after eating home-canned spinach. It was noted that the contents of the jar had a "bad scum" on the surface, but this was discarded and the remainder of the spinach "warmed up" and served. No unusual odor was detected when the spinach was heated, and no bad taste was noted.

All the patients developed symptoms within 24 hours after eating the spinach, and they died on the third, seventh and ninth days respectively.

No material was available for laboratory tests.

Reported to the commission.

B. Case Clinically Typical.

REPORT 49.—*Causative food unknown.*

In November, 1919, at Monroe, Wash., a man developed typical symptoms of botulism and died on the sixth day of illness. There was no record of what food was responsible for the poisoning, but commercially canned olives were suspected.

No material was available for laboratory tests.

Reported to the commission.

BOTULISM SURVEY IN THE YAKIMA VALLEY, STATE OF WASHINGTON.

During April, 1921, an outbreak of botulism from canned spinach, home prepared by means of a pressure cooker, occurred near Toppenish, Yakima County, Wash. At the request of the commission, this outbreak was investigated by Dr. C. R. Fellers, of the National Canners Association, attached to the laboratory of the Pacific Fisheries Investigations (Dr. E. D. Clark, director, Seattle, Wash.). The information submitted by Dr. Fellers as a result of his inquiry concerning the Toppenish outbreak was regarded of such importance that a complete survey of the Yakima suggested itself and was later carried out by a member of the commission.

The valley is geographically, in many respects, similar to certain parts of California. The country, though regarded as one valley and situated between rather high foothills of the Cascade Mountains, is really a series of three smaller valleys more or less constricted in area, which for convenience of a survey can be called the Tieton, the Yakima, and the Toppenish. The following geographical notes are of some interest:

Population, estimated	60,000
Total area (square miles)	4,112
Land under cultivation (acres)	140,000
Assessed valuation	$37,232,621

One hundred and thirty-two miles of graded and surfaced roads.

Live stock in country: Horses and mules, 16,000; stock cattle, 24,745; dairy cattle, 13,189; sheep, 138,608; hogs, 22,908; poultry, 172,980.

Mean annual precipitation, 8.35 inches, and snowfall 25.3 inches.

Fifty thousand acres of fruit trees, about one-half of which are in some state of bearing: 70 per cent apples, 10 per cent peaches, 10 per cent pears, and the balance prunes, cherries, plums, apricots, etc.

It is evident from this brief description that the Yakima Valley is one of the most productive and progressive agricultural and fruit-growing districts on the Pacific Coast.

According to the best available sources of information, there have occurred in the valleys during the last five years 7 outbreaks of human botulism, 27 outbreaks in domestic fowls, 26 in horses, 3 in cattle, and 1 in hogs. The mortality rate among the human cases was 50 per cent. Aside from being an important public-health problem, botulism assumes in the Yakima Valley also a noteworthy economic aspect, if one realizes that the loss in animals has been rather large, namely, 1,150 chickens, 143 horses, and 30 hogs. Inasmuch as the information collected extends only over a comparatively short period of time, there is no doubt that many other outbreaks have occurred and have been overlooked. The distribution of the outbreaks is shown in figure 6 and summarized in Table 6.

TABLE 6.

	Areas.		
	Yakima.	Toppenish.	Tieton.
Human	6	1	0
Chickens	10	1	16
Horses	5	12	6
Cattle and hogs	1	2	1

The human outbreaks which have occurred in Yakima are briefly abstracted below.

Cases Proven by Bacteriologic and Toxicologic Tests.

PLANT PRODUCTS.

REPORT 50.—*Corn, locally grown and home canned.*

In April, 1921, at Yakima, Wash., a woman tasted about one-half teaspoonful of home canned corn. She discarded the lot because it had a sour and biting taste. On the following day she developed typical symptoms of botulism and on the ninth day after tasting the corn she died.

Necropsy was not performed.

The corn was grown in the vicinity and canned by the cold-pack method. *B. botulinus*, type A, was isolated from the contents of another jar of the same pack.

Investigated by the commission.

REPORT 51.—*Corn, locally grown and home canned.*

In January, 1916, at Yakima, Wash., a woman swallowed about two teaspoonfuls of home-canned corn and discarded the remainder because it was spoiled. About 48 hours later she developed typical symptoms of botulism and died on the sixth day of illness.

Necropsy was not performed and no laboratory tests were made.

A number of chickens ate the discarded corn and all developed typical fowl botulism and died.

The corn had been grown in the vicinity and canned at home.

Reported to the commission.

REPORT 52.—*Corn, locally grown and home canned.*

In November, 1917, at Yakima, Wash. a woman opened a jar of corn which "had the top of the jar pushed out" and contained bubbles of gas. Notwithstanding the visible spoilage, she tasted a small amount and noted that it was bitter. Within two days she developed typical symptoms of botulism and died on the fourteenth day of her illness.

Necropsy was not performed.

A number of chickens consumed the discarded portion of the corn and all died of fowl botulism.

No laboratory tests were made.

Reported to the commission.

REPORT 53.—*Corn, locally grown and home canned.*

In December, 1918, at Yakima, Wash., a woman opened a jar of corn which was obviously spoiled. She tasted a portion of the corn and found it slightly sour. She boiled a portion of the corn for 30 to 40 minutes and while it was being heated she noted an unusual odor but nevertheless she gave it to two children. On the following day the mother developed typical symptoms of botulism but recovered, although there was transient blepharoptosis at times for nine months. The children did not show any signs of poisoning.

A number of chickens which consumed a portion of the corn which was discarded without having been cooked developed typical symptoms of botulism.

No laboratory tests were made.

The corn was grown in the vicinity and canned at home.

Reported to the commission.

REPORT 54.—*Asparagus, locally grown and home canned.*

In November, 1910, at North Yakima, Wash., a woman served "warmed" but not boiled home-canned asparagus at dinner. She had opened one jar and found it spoiled and with a bad odor but believed the next two jars to be good. Ten persons took asparagus on their plates, but as soon as the hostess tasted it she noted that it was spoiled and advised her guests to discard it. All but two of the party did so.

Of the 10 persons who had asparagus on their plates, it is known that 2 ate the whole portion, 2 men merely tasted the asparagus, and the remaining 6 ate only a portion of the gravy. Six persons, 2 men and 4 women, developed typical symptoms of botulism and 3 died. The 2 men who merely tasted the asparagus did not develop symptoms; an observation which was attributed to the fact that they both drank freely of beer after dinner. The onset in three cases was on the fourth, sixth, and eighth days after ingesting the poison. In the fatal cases, the illness lasted two or three days after the onset and in those which recovered there was no marked improvement for about three weeks.

Necropsies were not performed

A number of chickens which ate the discarded asparagus developed typical fowl botulism and died.

Reported by McCracken. The Leucocyte, Detroit, 1912, *16*, p. 80.

REPORT 55.—*Spinach, locally grown and home canned.*

In April, 1921, in the Toppenish area of Yakima, Wash., a man and his wife developed typical symptoms of botulism and died after eating home-canned spinach which showed no signs of spoilage and which tasted good. The onset of symptoms occurred on the first and second days, and death on the fourth and ninth days after ingesting the poison. In one case, the leucocyte count on the day of death was 16,000 and the urine was normal.

Necropsies were not performed and no laboratory tests for *B. botulinus* were made. A number of chickens which ate the discarded spinach developed typical fowl botulism.

The spinach was grown in the vicinity and canned at home in an aluminum pressure cooker. The recommendations as to processing which were issued by the manufacturers of the cooker were closely followed.

Investigated by the commission.

ANIMAL PRODUCTS.

REPORT 56.—*Milk, commercially canned.*

In October, 1920, at Yakima, Wash., four persons (a man and his wife and two children, aged 4 and 3) developed typical symptoms of botulism after consuming spoiled canned milk, which was mixed with coffee. The onset of symptoms in one case, the father, was 24 hours and in the other three, 48 hours after the poison was ingested. All the patients recovered.

In many of the cans the milk was thick and would not flow from punched holes. It was the custom of the family to use the thickened milk if it had no bad odor, and it was from such a product that the poisoning was apparently produced.

None of the contents of the suspected can was available for laboratory tests, but bacteriologic examination of the contents of other cans showed that there was much sediment, thought to be dirt or manure, and that they contained viable bacteria. *B. botulinus* was not isolated.

Investigated by the commission.

PLANT PRODUCTS.

REPORT 90.[2]—*Asparagus, locally grown and home canned.*

In October, 1921, at Walla Walla, Wash., an adult woman opened a 2-quart jar of home-canned asparagus and tasted twice a portion of the content. About 48 hours later she developed typical symptoms of botulism and died on the ninth day after the tasting of the toxic food. Necropsy was not allowed.

The asparagus was heated and the son of the deceased tasted the product. He noted a "cheesy taste" and spit out part of the piece he tasted but swallowed part of it. He had no ill-effects.

The vegetables were grown locally and were canned in one and two quart jars during May or June of 1921. No detailed account of the method of canning was available, because the woman did the work alone; one daughter stated that the usual method was cold pack, boiled three hours in an open boiler and sealed hot. No preservative was used in canning except the usual salt seasoning.

One 2-quart jar had been used and had produced no ill effects. No part of the contents of the original jar tasted was available and the jar containing the food was broken and placed in the fire. It was not known that the product was spoiled until the poisoning occurred. Of the remaining six jars on hand, four showed evidence of spoilage; either discoloration, swelling of the lids, odor or all combined. Three of these jars submitted for examination showed marked evidence of disintegration of the stalks, leaking lids and repulsive odor. They all contained *B. botulinus* toxin, type A; the

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[2] Reported since July 31, 1921.

M. L. D. varied between 1: 10,000–1: 50,000. *B. botulinus*, type A, was isolated directly from one jar in pure culture. Shake cultures of the liquor of the remaining jars gave numerous pure colonies of the specific organism. The liquor was still toxic after an exposure to a temperature of 80° for 30 minutes. The H-ion concentration of the toxic liquor of one jar (M. L. D. 1: 10,000) was pH 3.82 and of the two others pH 6.28 and 7.35.

The heated asparagus was thrown out to the chickens, 23 of which died out of a total flock of 35.

Investigated by Mr. R. T. Elliott, Seattle station, Bureau of Chemistry. Bacteriological and toxicological studies made by the laboratory of the commission.

REPORT 91.[3]—*Corn, home grown and home canned.*

In October, 1921, at Yakima, Washington, an adult woman opened a jar of corn and tasted a portion of the contents which had a bad odor and appeared spoiled. She developed typical symptoms of botulism and died on the fourth day after the onset of the symptoms.

Necropsy was not allowed.

The case was diagnosed as poliomyelitis by an osteopath, but later seen by a deputy health officer and reported as botulism.

Nine chickens which ate the discarded corn developed fatal fowl botulism. The crop of one of the chickens contained *B. botulinus* toxin, type A, and the organism was isolated in pure culture.

Reported by Dr. H. H. Smith, health officer of Yakima; bacteriological and toxicological tests made by the commission.

In a general way, the botulism observations made in the Yakima Valley differ in no respect from those reported for the entire State of Washington, or for California. It is therefore only necessary to point out certain interesting peculiarities. The case mortality rate for the 13 outbreaks in Washington was 69.1 per cent, namely 18 deaths among 26 cases. Again botulism was only observed during the fall, winter, and spring months, from October until May; one-third of all the cases occurred during the month of November. The customary habit of consuming preserved vegetables during the seasons in which the fresh products are not available explains these facts.

TABLE 7.

Report.	Food.	Canning processes.			Spoilage.	Cases.	Amount consumed.	Laboratory proof.	Animals affected.
		Home.	Commercial.	Method.					
50	Corn	–		Cold pack.	+	1	2 teaspoonfuls.		Chickens.
51	do	+		do	+	1	Tasted		Do.
52	do	+		do	+	1	do		Do.
53	do	+		do	+	1	Teaspoonful	+, type A.	
48	Spinach	+		do	±	3			
55	do	+		Pressure cooker.	No.	2			Do.
47	Asparagus	+		Cold pack.	+	3		+, type A.	Do.
54	do	+		do	+	6			Do.
46	String beans	–		do	+ +	1	3–6 small bean pods.	+, type A.	
56	Milk		+	Pressure	+	4			

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[3] Reported since July 31, 1921.

The foodstuffs which were responsible for the botulism cases in Washington are graphically shown in figure 5, and the various factors accompanying the poisoning cases are summarized in Table 7. Ten of the reports state that home-canned vegetable food was responsible for the intoxications. In four of the seven instances in which single cases occurred the habit of tasting visibly spoiled food led to fatalities among housewives. Instead of string beans, corn was the most frequent cause. This vegetable is excellently suited for the development of a very potent poison and offers extreme difficulties in sterilization on account of the slow heat penetration in heavily filled jars. Furthermore, it is not unlikely that the repeated handling, which is necessary to prepare this vegetable for canning, assists in the contamination of the product with the spores of *B. botulinus*.

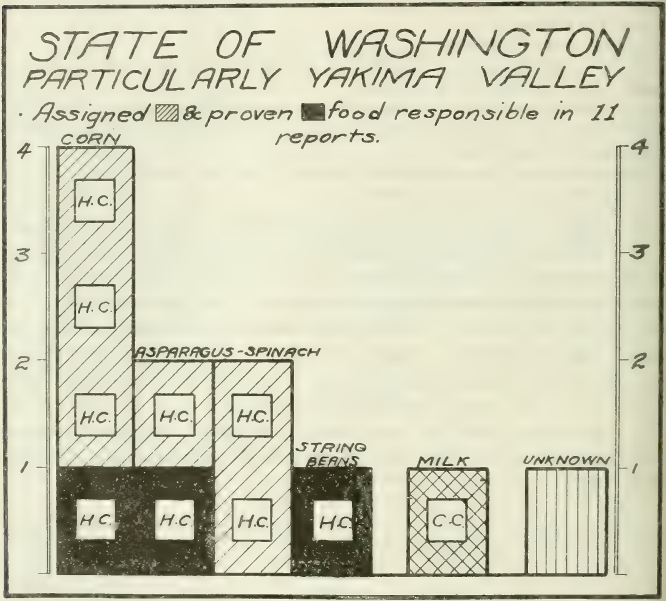

Fig. 5.

Spoilage of the causative food was recognized and admitted in all but one instance. In this particular outbreak, spinach which had been processed in a pressure cooker was assigned as the source of the poison. This observation lends additional support to the statement previously made that the degree of macroscopic spoilage of the preserved spinach in the form of changes in consistency and odor may be so slight as to escape the attention of the cook or consumer. Health officers, teachers in home canning and other agencies should direct their attention to these facts and proclaim their importance with unremitting insistence.

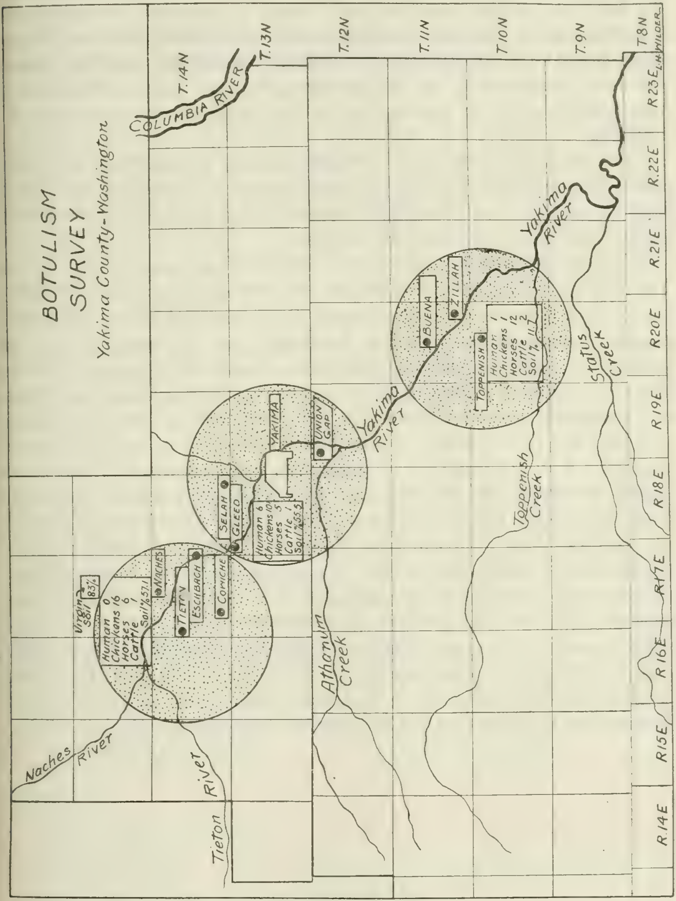

Fig 6.

Unfortunately, there were neither toxicologic nor bacteriologic tests to establish the responsibility of the evaporated milk, but the epidemiological data were so convincing as to leave little ground for doubt. Recent laboratory tests have shown that *B. botulinus* in pure culture does not grow freely in evaporated milk and it must be assumed that if the milk contained the toxin, there must have been some contributary factor in this case. There was no opportunity of investigating the contents of the can which is supposed to have caused the poisoning, but cans of the same lot were found to contain a sediment resembling dirt or manure, a condition of affairs which makes it obvious that the product would be difficult to sterilize and that there was every opportunity for the presence of other bacteria which may have played a rôle in making the milk suitable for the growth of *B. botulinus*.

In view of this outbreak, the milk-canning industry can not consider its products immune to the formation of the botulinus toxin and they should at once establish methods of procedure which would insure absolute cleanliness and sterility.

A bacteriological study of over 100 soil specimens collected in Yakima Valley explains the rather frequent development of *B. botulinus* in underprocessed home-canned vegetables and fruits. The percentage figures shown in figure 6 suggest that *B. botulinus* is in all probability an inhabitant of virgin soil. It may be mere coincidence due to methods of sampling but the available data indicate that from the mountain ranges toward the valleys following the descending course of the river, there is a progressive reduction in the infection of the soil. For example, virgin mountain soil contained *B. botulinus* spores in 83 per cent of instances, while earth collected in the adjacent Tieton and Yakima Valley sections gave 57.1 per cent and 55.5 per cent cultures. On the other hand, the Toppenish area, which is further removed from the source of the Yakima River, supplied comparatively few infected soil specimens (11.7 per cent). These facts suggest a number of important questions which can only be answered when intensive laboratory studies already in progress have been completed. However, observations made in California and Wyoming confirm the data collected in Yakima. They seem to indicate that *B. botulinus* is rather a common anearobe in mountain soil

Part V.

HUMAN BOTULISM IN THE UNITED STATES AND CANADA, EXCLUSIVE OF THE STATES OF CALIFORNIA AND WASHINGTON.

The commission has collected all the reports dealing with botulism which were accessible in the medical literature and daily press. A limited number of recent outbreaks were investigated by the commission and are reported here for the first time. In some instances personal communications of health officers have amplified the published reports. For the sake of brevity, the various reports are abstracted and only the most essential points which deserve discussion are mentioned.

A. Single Cases or Those Occurring in Groups and Families, Proven by Bacteriological and Toxicological Tests.

(1) PLANT PRODUCTS.

(*a*) Ripe Olives.

Report 57.—*Ripe olives, commercially bottled.*

In August, 1919, at Canton, Ohio, ripe olives were served at a table at which 18 persons were seated. It had been noted by a waiter that the vacuum of the container was lost and that the olives did not "taste right" and several of the guests remarked that they "smelled like limburger," "bit the tongue," "seemed to pucker the mouth," etc. Fourteen of the guests, the chef, and two waiters ate or tasted a portion of the olives and all but three developed typical symptoms of botulism. In general, it appeared that the severity of the intoxication and the rapidity of deaths was directly referable to the amount of olives ingested.

The 14 victims were all adults, 8 men and 6 women. Four men and 3 women died. In 1 case the onset of symptoms occurred within a few hours, in 6 on the first day, in 3 on the second day, in 2 on the third day, and in 1 on the fourth day after the olives were eaten. Four victims died on the third day, 2 on the fourth, and 1 on the eighth day after ingestion of the poison.

Necropsy was performed in two cases, but the viscera were examined only for chemical poisons.

It was noted that the two men who recovered had imbibed freely of whisky, and it is suggested that their survival might have been due to the action of the alcohol in destroying the toxin.

The olives were of the same pack as were responsible for outbreaks 58 and 60. Bacteriological investigation in several laboratories revealed the presence of botulinus toxin in the brine and viable *B. botulinus*, type A.

Reported by Armstrong, Story, and Scott. Public Health Reports, 1919, *34*, p. 2877.

REPORT 58.—*Ripe olives, commercially bottled.*

In October, 1919, at Detroit, Mich., ripe olives of the same pack as caused outbreak 57 were served at dinner. One of the guests remarked that the olives were soft and had a peculiar odor, but nevertheless four of the diners and one servant ate some of them. During the following three days, the son, aged 12, an adult daughter, and a waitress consumed the greater part of the olives; five women, one man, and one boy developed typical symptoms of botulism and three women, the man, and the boy died. One person who ate some of the olives escaped illness.

In only three cases was the consumption of olives limited to a single occasion so that the time of onset could be estimated, and of these one showed first signs of illness within 12 hours, one within 24 hours, and one, a mild case, 65 hours after ingesting the poison. One patient died in 24 hours, one in three days, two in four days, and one between five and six days after the first of the olives was eaten.

Necropsies are not reported.

Botulism antitoxin was administered on the fifth day to two patients, one who died and one who recovered, but subsequent tests proved that the type of antitoxin was not homologous with that of the toxin that caused the poisoning.

Portions of the olives and the brine were sent to several laboratories for examination and *B. botulinus*, type A, was demonstrated in all. A virulent toxin was also demonstrated in the brine.

Reported by Jennings, Haas, and Jennings, Jour. A. M. A., 1920, *74*, p. 77, and Emerson and Collins, Jour. Lab. and Clin. Med., 1920, *5*, p. 559.

REPORT 59.—*Ripe olives, commercially bottled.*

In January, 1920, at New York City, a woman opened a jar of ripe olives and presumably ate several. On the evening of January 9, she began to vomit and the next day she showed typical symptoms of botulism. She died the following day about 14 hours after the onset of symptoms.

Necropsy was not performed.

At the evening meal on January 13 a salad was served which contained ripe olives from the same bottle, and it was stated that several members of the family had eaten some of the olives while the salad was being prepared. The man who prepared the salad said that he had noticed nothing wrong with the taste or odor, but had found them "very good." Six persons (three men, two boys, and one girl) ate either the salad or whole ripe olives, or both, and all but the girl developed typical symptoms of botulism and died. The girl insisted that she had eaten none of the salad and only one olive, which tasted good. The onset of symptoms occurred 4, 22, 26 (two patients), and 50 hours after the food was eaten and death occurred 24½, 72, and 74 hours, respectively, after the onset of symptoms.

Botulinus antitoxin was administered intravenously in one case without beneficial effect. It was subsequently shown that the type of the antitoxin was different from that of the toxin which caused the poisoning. Antitoxin was also given as a precautionary measure to the girl who had not developed any symptoms of poisoning.

Necropsy was performed in one case but the details of the examination are not recorded.

The olives had been in New York since October, 1917. No evidence of spoilage was detected at the time the olives were consumed, but it is stated by the author of the report that in the laboratory it was noted that "the odor had a weak but apparently quite definite putrefactive tinge," although "there was considerable controversy in the laboratories in New York and Boston as to whether the odor present in the olives could be considered characteristic enough to be of protective value to the average individual."

B. botulinus, type A, was isolated from the olives remaining in the container and botulinus toxin was demonstrated in the liquid.

Reported by Sisco. Jour. A. M. A., 1920, *74*, pp. 516, 690.

Report 60.—*Ripe olives, commercially bottled.*

In February, 1920, at Memphis, Tenn., ripe-olive sandwiches were served at an afternoon party. Seven persons (two men, four women, and a boy) ate some of the olives and all died. The onset of symptoms occurred within 24 hours in four cases, on the second day in two cases, and on the third day in one. Two of the victims died on the second day, two on the third, two on the fourth, and one on the ninth day after ingesting the poison.

Necropsies are not recorded.

The olives were of the same pack as caused outbreaks 58 and 59. It is not noted that spoilage was detected. *B. botulinus*, type A, was isolated from the olives which remained.

Reported by Fontaine. Jour. A. M. A., 1920, *74*, p. 470. Further data obtained from the Health Department of Memphis.

Report 61.—*Ripe olives (stuffed with pimento), Commercially bottled.*

In November, 1919, at Java, Mont., seven persons (four men, two women, and one little girl aged 5 years), ate stuffed ripe olives and all developed typical symptoms of botulism. Three men and two women died. The onset of symptoms occurred within 24 hours in two cases, on the second day in four, and on the third day in one. Death occurred on the third day in two cases, on the eighth day in two, and on the ninth day in one.

Necropsies were not performed.

The olives had been relabeled by the distributer. The only material which was available for laboratory tests was the glass container which was recovered on November 23, after being exposed in the snow. The United States Department of Agriculture reports that *B. botulinus*, type A, was isolated from dried pimento which was adherent to the glass.

Reported by De Bord, Edmonson, and Thom. Jour. A. M. A., 1920, *74*, p. 1220.

Report 62.—*Ripe olives, commercially bottled.*

In June, 1921, at Greensburg, Pa., five persons ate ripe olives which, according to the statement of two of the victims and of the cook, did not appear to be spoiled. Two women who ate one olive each did not show signs of serious illness although one vomited after the meal, but three women developed typical symptoms of botulism and died. It is stated that the onset of symptoms in two cases occurred within 24 hours and that death occurred on the second, third, and fifth days, respectively.

Necropsies were not recorded.

Botulinus antitoxin (type unstated) was administered on the fourth day to one patient without beneficial effect. This patient was also kept under anæsthesia for three days prior to death.

It was stated in one laboratory that the olive brine was cloudy and had a "musty, rotten, molasses odor."

B. botulinus, type A, was isolated.

From the official report to the Bureau of Chemistry, U. S. Department of Agriculture.

(*b*) Spinach.

Report 63.—*Spinach, commercially canned (processed by boiling for three hours).*

In January, 1921, at Grand Rapids, Mich., 29 persons (23 women and 6 men) developed typical symptoms of botulism and 3 died. The cause of the poisoning was subsequently shown to be commercially canned spinach which is said to have had no unusual odor or indication of spoilage although the taste was "biting." The onset of symptoms in 18 cases was within 24 hours, in 5 cases on the second day, in 2 on the third day, in 1 on the fourth, and in 2 on the sixth day after eating the spinach. Death occurred in 2 cases on the third day and in 1 on the fourth day after ingesting the poison.

Necropsy was performed in all fatal cases but the details of the examination are not yet recorded.

The spinach was of a pack which was processed at a temperature considerably below that of the ordinary trade procedure and in which there was a high per cent of spoilage. None of the original spinach was available for test, but *B. botulinus*, type A, was recovered from the contents of another can of the same lot. *B. botulinus*, type A, was also isolated from the stool of one of the victims which was collected on the eleventh day after the spinach was eaten, but in another stool collected on the thirty-first day no *B. botulinus* was detected.

Reported by Wells. Jour. Mich. State Med. Soc., 1921, *20*, p. 381. Additional data were given by Doctor Wells in a personal communication to the commission.

Report 64.—*Spinach, commercially canned.*

In January, 1921, at Battle Creek, Mich., five women developed typical symptoms of botulism and two died. It is stated that no common article of food could be traced and that "the only suspicious food was canned spinach, which was shown in a few instances to be infected, giving positive tests to type A."

There are no accurate data concerning the time of onset of symptoms but in the two fatal cases death occurred on the third and eighth day of illness.

Necropsy was performed in one case. It is stated that "colonies of bacilli which seemed to be *B. botulinus* * * * were noted in the walls of small gas cysts located in the pons and medulla."

The spinach was of a pack which had been processed below the standard of the trade. *B. botulinus*, type A, was recovered from some of the cans.

Reported by Colver. Jour. Mich. State Med. Soc., 1921, *20*, p. 385.

Report 65.—*Spinach, commercially canned.*

In February, 1921, at Mischawaka, Ind., two persons (a man and a woman) developed typical symptoms of botulism and died after eating canned spinach which was said to have shown no signs of spoilage and to have tasted normal. The onset of symptoms occurred within 24 hours and the patients died on the fifth day after eating the spinach.

Necropsies were not reported.

Antitoxin (type unknown) was administered without beneficial result.

Bacteriologic examination of the spinach revealed *B. botulinus*, type A, and virulent toxin.

Reported to the commission by the attending physician.

(c) Corn.

Report 66.—*Corn, locally grown and home canned.*

In October, 1915, at Hillsboro, Oreg., a woman opened two jars of corn which were obviously spoiled and had an offensive odor. She then opened a third jar which she thought smelled good, but when she tasted about one teaspoonful she decided it also was spoiled and discarded the lot. In about four hours she noted pain in the abdomen and on the following day she developed typical symptoms of botulism. She died on the fifth day after eating the corn.

Necropsy was performed but no cause of death was determined.

The corn had been canned at home several months previously. A number of hogs and chickens consumed that which was discarded and 50 chickens developed typical symptoms of fowl botulism and died. The hogs showed no signs of illness.

B. botulinus, type A, was isolated from the contents of the gizzard of one of the chickens.

Reported by Dickson. Monograph of the Rockfeller Institute, No. 8, 1818, p. 24.

(*d*) ASPARAGUS.

REPORT 67.—*Asparagus, home canned.*

In January, 1918, at Boise, Idaho, a salad prepared from cold home-canned asparagus was served at dinner. When the jar was opened, one of the guests remarked that it smelled spoiled, but the hostess replied, "It can't be; I canned it myself." One victim noted that it did not taste right.

Five persons partook of the salad and four developed typical symptoms of botulism and died, one woman, while being moved to the hospital in an ambulance. The onset of symptoms in three cases is given as within 18 hours after the food was eaten. Two persons died within 24 hours after the meal, and two others died within 48 hours after ingesting the poison. One man who "ate heavily of asparagus" escaped illness.

Necropsies are not recorded.

B. botulinus, type A, was isolated from the remnants of the salad and asparagus juice and extra cap of a container.

Reported by Thom, Edmonson, and Giltner. Jour. A. M. A., 1919, *73*, p. 907.

(*e*) BEETS.

REPORT 68.—*Beets, commercially canned.*

In May, 1920, at Florence, Ariz., five men (three Indians and two white men) ate uncooked commercially canned beets which were apparently not spoiled. All developed typical botulism symptoms and died. The onset of symptoms in four cases occurred within 24 hours and in one case within 48 hours after the poison was ingested. One man died on the second day, two on the third, one on the fourth, and one on the seventh day after the meal.

Necropsy was performed in one case and microscopic examination showed prethrombitic foci in the vessels of the brain.

Botulinus antitoxin, types A and B, was administered by intravenous injections in one case on the fifth day after the food was eaten, without beneficial results. Seventy cc. were injected.

B. botulinus, type B, was recovered from the wall of the jejunum of one of the victims who died.

Reported by W. E. Randall. Jour. A. M. A., 1920, *75*, p. 33. Also investigated by the commission and referred to in Public Health Reports, 1921, *36*, p. 1313.

(*f*) STRING BEANS.

REPORT 69.—*String beans, home canned.*

In February, 1918, at Decatur, Ind., two men, four women, and one boy ate home-canned string beans and all developed typical symptoms of botulism. There is no record that the beans were spoiled. The onset of symptoms in two cases occurred within 12 hours, in four within 24 hours and in one two days after the meal. The four deaths occurred on the fourth, sixth, seventh, and ninth days, respectively.

Homologous antitoxin was administered to three victims, beginning a little over eight days after the ingestion of the poison. One of the patients died a few hours after the injection and the other recovered.

Bacteriologic examination of the contents of the jar showed that the juice surrounding the beans did not contain virulent toxin, but *B. botulinus*, type B, was recovered from the spleen of one of the victims. (Graham.)

It is not recorded that any chickens ate of the beans, but in the Decatur Democrat of March 1, 1918, it is stated that a hog which had been fed garbage from the place where the poisoning occurred had developed signs of paralysis and died.

Reported by McCaskey. Amer. Jour. Med. Sci., 1919, *158*, p. 57. Additional data contained in a personal communication from Professor Graham, Urbana, Ill.

(2) ANIMAL PRODUCTS.

(*a*) HAM.

REPORT 70.—*Ham, preserved at home.*

In 1916, at Tampa, Fla., seven men developed typical symptoms of botulism and all died after eating a native "razorback ham, which did not have any bad appearance or odor." The onset of illness in all cases occurred within 24 hours after the ham was eaten; one man died within 18 hours and none lived beyond five days after ingesting the poison.

A portion of the ham was macerated in sterile salt solution and the liquid given to a cat. The cat developed typical symptoms of botulism and died. A similar result was observed in a dog to which a small portion of meat was fed. A mule was fed four ounces of tube and plate culture without effect. *B. botulinus* was recovered in pure culture from the ham.

Reported by Randall. Medical Record, 1920, *98*, p. 763.

(*b*) SAUSAGES.

REPORT 71.—*Blood sausage, prepared at home.*

In 1912, at Lowell, Mass., two men, one woman, and one girl developed typical symptoms of botulism after eating homemade blood sausages, which were "boiled in water" before they were eaten. The onset of symptoms in all cases was within 24 hours after the meal. One of the victims died on the third day and one on the fourth and two on the fifth day after ingesting the poison.

Necropsies were not allowed.

The sausage was prepared from pork scraps and was packed into a casing prepared from the large intestine of a hog. *B. botulinus* was recovered from the sausage.

Reported by E. M. Randall. Medical Record, 1920, *98*, p. 763.

REPORT 72.[4]—*Summer sausage, commercially prepared.*

In December, 1919, at Milwaukee, Wis., three persons (two men and one woman) became ill after eating summer sausage, which had been purchased from a commercial packer. There is no record of any indication of spoilage. About one-half hour after the meal "all three became ill with nausea, vomiting, and gastrointestinal symptoms. The acute symptoms lasted for one day, gradually subsiding towards the end of the the third day."

A cat which ate the casing of the sausage showed similar symptoms, but recovered. On bacteriologic examination of the sausage, a spore bearing bacillus was isolated which was believed to be *B. botulinus*, but the recorded data concerning its formation of toxin are not convincing.

Reported by Church. Wisconsin Med. Jour., 1919–20, *18*, p. 377.

(c) CHEESE.

REPORT 73.—*Cottage cheese, home prepared.*

In October, 1914, three persons became ill and died after eating homemade cottage cheese which had been stored in a tin bucket in the cellar. All showed typical symptoms of botulism.

Necropsy was performed in one case and was reported negative for poliomyelitis.

An emulsion of the cheese after incubation for 48 hours killed guinea pigs in 36 hours and *B. botulinus*, type B, was recovered in cultures from the cheese. It is stated that the cheese had a slight putrefactive odor due to the presence of *B. coli*.

Reported by Nevin. Jour. Infect. Dis., 1921, *82*, p. 226. Additional data were given the commission by the New York State Board of Health.

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[4] The symptoms of the patients and the short duration of illness are not typical of botulism, and this fact, together with indefinite results of animal inoculation, suggests the possibility that the diagnosis of botulism was not fully justified.

B. Clinically Typical Cases, Single or in Groups and Families, Causative Food Assigned on Epidemiological Grounds Only.

(1) PLANT PRODUCTS.

(a) String Beans.

Report 74.—*String beans, commercially canned.*

In 1915, at Basalt, Colo., five persons died after eating string beans. The symptoms were those of botulism. No clinical or laboratory data were available

Reported by Curfman. Colorado Medicine, 1917, *14*, p. 35.

Report 75.—*String beans or spinach, commercially canned*

In October, 1912, at Romley, Colo., six men and one woman developed typical symptoms of botulism and five of the men died, after eating canned string beans or spinach. The onset of symptoms varied from about eight hours to four days after the ingestion of the poison, and the deaths occurred in the fatal cases on the second, third, sixth, and fourteenth days after the meals. Convalescence of the two who recovered was prolonged and tedious.

Necropsy in one case showed congestion of the viscera, and thick mucus in the bronchi. Remnants of the beans and spinach were consumed by five burros, all of which developed typical symptoms of botulism and died. The contents of another can of spinach of the same lot was fed to a Belgian hare without producing symptoms.

Reported by Curfman. Colorado Medicine, 1917, *14*, p. 35.

Report 76.—*String beans, home canned.*

In December, 1917, at Ontario, Oreg., a woman opened a jar of string beans and tasted the contents to determine whether they were good. She decided they were "not exactly right" and discarded them. On the following day she developed typical symptoms of botulism and died about 52 hours after tasting the beans.

Necropsy was not performed.

A number of chickens ate the discarded beans and 39 of them died of fowl botulism within two or three days.

Reported by Dickson. Arch. Int. Med., 1918, *22*, p. 483.

Report 77.—*String beans, locally grown and home canned.*

In October, 1918, at Klamath Falls, Oreg., one woman tasted a portion of home-canned string beans which were obviously spoiled. Within 24 hours she developed typical symptoms of botulism and died on the fifth day after ingesting the poison.

A number of chickens ate the discarded beans and all died of typical fowl botulism.

No laboratory tests were made.

Reported to the commission.

Report 78.—*String beans, home canned.*

In March, 1918, at Newark, N. J., three persons became ill and two died after eating home-canned string beans as salad. The symptoms were typical of botulinus intoxication.

A number of chickens which ate the discarded beans died of typical fowl botulism.

Investigated by G. C. De Bord, Bureau of Chemistry, U. S. Department of Agriculture. Mentioned by Thom, Edmonson, and Giltner, Jour. A. M. A., 1919, *73*, p. 912, footnote 20.

(b) Corn.

Report 79.—*Corn, locally grown, home canned.*

In November, 1917, in New York State, seven persons (two men, one woman, and four children) ate succotash made from home-canned corn and fresh beans. One adult visitor refused to eat the vegetable because of the bad odor, and some who ate it are said to have pronounced it "bad." One person merely tasted it. The seven who ate the succotash developed typical symptoms of botulism and five died. The onset of symptoms occurred in four cases, three children and one adult, within 24 hours, and in one child on the second day after the meal. One of the victims died on the third day, three on the fourth, and one on the fifth day after ingesting the poison.

Necropsy was performed in one case and congestion of the viscera and hemorrhages of the intestines were noted.

No material was available for laboratory tests.

Reported to the commission.

Report 80.—*Corn, home canned.*

In January, 1920, at Pittsburgh, Pa., two men, one woman, and one boy developed typical symptoms of botulism after eating home-canned corn which tasted sour. The onset of symptoms in all cases was within 24 hours after the meal. Three of the victims died, one child on the third day, one adult on the eighth day, and one on the ninth day after ingesting the poison.

Clinical laboratory tests are reported as follows: Leucocyte count in one case, 6,700, with 71 per cent polymorphonuclear neutrophils, and in another, 8,300, with 73 per cent polymorphonuclears; spinal fluid in one case taken during life showed 3 cells per c. mm.; globulin test negative and Wassermann test negative; blood pressures registered 116/85 and 108/80 in two cases.

Blood serum of one patient was injected into animals without producing symptoms.

Necropsy was performed in all three cases and on microscopic examination the brains of two were negative but the brain of one showed hemorrhagic areas in the medulla and the lung of another contained hemorrhagic infarcts.

Anaerobic cultures from the liver, spleen, and feces in three cases, from the lungs and heart's blood in two, and from the brain in one were negative for *B. botulinus.*

Bacteriologic examination of the contents of another jar of the same pack showed viable spore bearing bacilli but no *B. botulinus.*

Reported to the commission by Drs. S. R. Haythorn and E. R. Lacy, William H. Singer Memorial Research Laboratory, Pittsburgh, Pa.

(*c*) Beets.

Report 81.—*Beets, home canned.*

In April, 1921, at Pueblo, Colo., two men, two boys, aged 13 and 9, and one girl aged 4, developed typical symptoms of botulism, and three of them died. The onset of symptoms in two cases, both of whom died, was within 24 hours, in two, one of whom recovered on the second day, and in one mild case on the third day, after the suspected food was eaten. The blood count in one case showed R. B. C. 4,850,000, leucocytes 8,800, polymorphonuclear neutrophiles 80 per cent.

Necropsies were performed, but the examinations have not been recorded in detail. In one case there was purulent nasopharyngeal discharge, hyperemia of the left hemisphere, and softening of the frontal lobe of the brain. Microscopic examination is not recorded.

Bacteriologic examinations of the brain of the victims were negative for *B. botulinus*, as was also a stool of a survivor, taken on the twenty-sixth day after the meal.

There was some question as to the cause of the poisoning, and the following table gives the details of the menu and the amount of various foods consumed by each person:

TABLE 8.

Name.	Age.	Chicken.	Potatoes.	Commercial canned corn.	Home-canned beets.	Dessert.	Status and treatment of case.
R. M	28	+	+	+	+	+	Mild case; 20 cc. of serum, subcutaneously.
H. M	13	+	+	+	+	+	Died.
F. M	9	+	+	0	(1)	+	Recovered; 40 cc. of serum, subcutaneously.
Mrs. M	25	+	+	0	0	+	Well.
Mrs. E	26	+	+	+	0	+	Well; suspicious symptoms 6 days later; given 20 cc. of serum subcutaneously.
A. E	4	+	+	+	+	+	Died.
J. E	6	+	+	+	0	-	Well.
Mr. E	29	+	+	+	+	+	Died.

[1] Beet.

There can be no suspicion of the canned corn, because it was in a normal-appearing can, had no bad taste or odor and no appearance of spoilage and was cooked for at least 30 minutes before it was served. Moreover, the housewife tasted the corn before it was cooked and did not develop symptoms. The beets had been grown and canned at home by the cold-pack method and were the last of a lot of 12 jars, 11 of which had been consumed. No spoilage had been detected in any of them. They were contained in the half-gallon jar, and it was noted that the liquid was cloudy, but this was poured off and replaced by hot vinegar. The jar was not a swell, and no unusual odor was detected, even when the hot vinegar was added. One of the survivors, an adult, declared that the taste was remarkably good.

Investigated by the commission. Data pertaining to the post-mortem material incomplete, because the specimens for examination were lost in the Pueblo flood.

REPORT 82.[5]—*Possibly beets, commercially canned.*

In May, 1919, about 20 miles from Dawson City in the Yukon territory, Canada, 23 persons developed typical symptoms of botulism and 12 died after a meal at which Government-inspected cold-storage beef, commercially canned corn, beets, apricots, pears, and milk were served. In 10 cases the onset of symptoms occurred within 24 hours, in 5 on the second day, in 2 on the third day, in 2 on the fourth day and in 1 on the fifth day after the meal. One of the patients died on the second day, 1 on the third, 3 on the fourth, 3 on the fifth, and 1 each on the sixth, nineth, eleventh, and fourteenth days after the food was eaten. Of the survivors, 8 were seriously ill and 3 were mild cases.

There was no material for bacteriologic examination as the Northwest Mounted Police disposed of the meat and the camp surgeon was one of the victims and unable to make a personal investigation at the time. It was supposed that the meat was responsible for the poisoning, because all of the victims ate meat, some of it quite rare, whereas it was recorded that two victims had not eaten beets. However, there is evidence that at least some of the beets had a peculiar taste. The fact that all who ate the beets did not become ill could be explained by the fact that the contents of five tins were served.

Reported by Glancy (one of the victims.) Canadian Med. Assoc. Jour., 1920, *10*, p. 1027.

(*d*) TOMATO CATSUP.

REPORT 83.—*Tomato catsup, commercially bottled.*

In Cook County, Ill., two persons, man and wife, developed symptoms of botulism and were ill for several weeks although they ultimately recovered. Bacteriologic examination of the suspected catsup yielded an anaerobic spore-bearing toxin-forming bacillus which gave positive tests for *B. botulinus.* It was not typed.

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[5] In the official report of these cases, it is stated that the cold-storage meat was believed to be the cause of the poisoning, but in the records it is noted that the canned beets had an unusual taste.

Personal communication by Graham, University of Illinois.

(*e*) SPINACH.

REPORT 84.—*Spinach, commercially canned.*

In April, 1920, in New York City, two persons, man and wife, developed typical symptoms of botulism and died. The onset of symptoms in both cases was within 24 hours after eating canned spinach in a restaurant. The husband said the spinach tasted good but the wife said her portion was "rotten." The woman died on the third day and her husband on the fourth day after ingesting the suspected food.

Botulinus antitoxin, "Nevin strain," type B, was administered to one case without beneficial result; 180 cc. were given intravenously.

Bacteriologic examination of the feces of the patients showed no *B. botulinus.*

No evidence of spoilage was detected in the spinach but it was of the same brand as that which caused the poisoning in outbreak 3. The spinach was removed from the can, cooked and kept warm by steam baths. It was stated by the proprietor of the restaurant that 18 other persons had been served spinach the same day and that none became ill.

Reported to the commission.

(2) ANIMAL PRODUCTS.

(*a*) CHICKEN.

REPORT 85.—*Minced chicken.*

In May, 1913, at Boston, Mass., one man developed typical symptoms of botulism from which he recovered after a tedious illness of more than six weeks. The onset of acute gastrointestinal disturbances occurred about two hours after ingestion of the suspected food. Minced chicken eaten in a restaurant was suspected of being responsible for the poisoning.

Laboratory tests were not made.

Reported by Stiles (the victim). Jour. A. M. A., 1913, *61*, p. 2301.

(*b*) HAM.

REPORT 86.—*Ham, commercially prepared.*

In June, 1920, at a summer resort in Maine, four persons (three men and one woman) developed typical symptoms of botulism and all died. The time of onset of symptoms is not stated, but the patients died on the third, fifth, ninth, and tenth day of illness, respectively.

The cause of poisoning was not determined, but it is known that all ate from the small end of a ham which had been purchased in the open market. Three of the victims stated that the ham had a bad taste.

Bacteriologic tests are not recorded.

Reported by E. M. Randall. Med. Record, 1920, *98*, p. 763.

REPORT 87.—*Smoked ham and salted pork, home prepared.*

"During November, 1919, at Sioux Rapids, Iowa, five persons became sick from eating smoked ham and salted pork kept in a barrel." Three of the patients died. In one case, which is recorded in detail, the patient was ill for over six weeks with an illness associated with fever as high as 104.2° F., and various symptoms of intracranial irritation, which are not characteristic of botulism.

Necropsy was performed, the anatomical diagnosis including fibrino-purulent basilar leptomeningitis, marked anemia and edema of the brain and anemia of the leptomeninges. Microscopic examination showed marked perivascular infiltration with lymphocytes.

Bacteriologic examinations of the exudate at the base of the brain and from the spleen were negative for *B. botulinus.* Cultures and feeding tests from the pieces of pork which remained in the barrel were negative for *B. botulinus.*

In view of the unusual clinical course and the character of the pathologic findings, as well as the absence of confirmatory bacteriologic evidence, there is justifiable doubt as to the correctness of the diagnosis of botulism in the case recorded.

Reported by Semerak. Jour. Infect. Dis., 1921, *29*, p. 190.

(3) CAUSATIVE FOOD UNKNOWN.

REPORT 88.—*Causative food unknown.*

In May, 1916, at Boston, Mass., a woman developed symptoms which strongly suggested botulism. After a tedious illness of several weeks she recovered. A diagnosis of botulism was made, but the carrier of the poison was not detected.

Reported by Lancaster. Trans. Amer. Ophthal. Soc., 1916, *14*, p. 648.

REPORT 89.—*Causative food unknown.*

In April, 1918, in New York City, one man developed symptoms which were diagnosed as botulism. He recovered after an illness of several weeks. The carrier of the poison was not detected.

Examination of the cerebrospinal fluid showed no increase in cells.

Reported by Grossman.' Neurological Bulletin, 1918, *1*, p. 260.

Since 1912, 33 instances of botulism have been reported for the entire United States and Canada, exclusive of the States of California and Washington. A total of 187 people, with 114 deaths, are involved in these observations. According to Table 9, the majority of cases occurred during the last five years. Furthermore, the annual number of outbreaks definitely increased during and immediately after the war, which prompted the investigations on which this report is based.

TABLE 9.

	United States and Canada, exclusive of California and Washington.						California.		Washington.		
	Home preserved.			Commercially preserved.							
	Bacteriologically proven.	Bacteriologically not proven.	Total.	Bacteriologically proven.	Bacteriologically not proven.	Total.	Home preserved.	Commercially preserved.	Home preserved.	Commercially preserved.	Total.
1910	0	0	0	0	0	0	1	1	1	0	3
1911	0	0	0	0	0	0	0	0	0	0	0
1912	1	0	1	0	1	1	2	1	0	0	5
1913	0	1	1	0	0	0	1	1	0	0	3
1914	1	0	1	0	0	0	0	0	0	0	1
1915	1	0	1	0	1	1	2	2	0	0	6
1916	1	0	1	0	0	0	2	0	1	0	4
1917	0	2	2	0	0	0	3	2	2	0	9
1918	2	2	4	0	0	0	6	1	1	0	12
1919	0	1	1	3	2	5	3	0	1	0	10
1920	1	1	2	2	1	3	2	3	0	1	11
1921 until June 30	0	1	1	4	2	6	1	2	5	0	15
Total	7	8	15	9	7	16	23	13	11	1	....
	Or a total of 31 outbreaks. +2 unknown.						Total 36. +3 before 1910. +6 unknown.		Total 12. +1 unknown.		
Home preserved											48
Commercially preserved											31
Total											79

In contrast to the observations made in California and Washington, only 6 histories refer to single cases, while 25 reports consider outbreaks with 2 to 29 cases of botulism. The predominance of commercially canned foods, as olives and spinach, which were served at dinner parties, may account for these differences. It is, however, more likely that many single cases have escaped recognition and are therefore not available for consideration.

AGE AND SEX DISTRIBUTION.

As previously stated, there is no evidence of any special incidence of either sex or at any age period. The sex distribution can be summarized by the following formula:

$$\text{Children : Males : Females} = 1:9.1:7.1.$$

INTOXICATION RATE.

It is again definitely shown that the intoxication rate is very high. In five outbreaks, however, either one or several persons, who ate of the poisonous food, escaped without showing symptoms. The histories of outbreaks 58, 59, and 62 state that single individuals, each consuming one olive, failed to contract botulism. Report 67 mentions that one negro, who ate heavily of the asparagus, escaped illness. There is some dispute relative to the number of persons who consumed the commercially canned beets responsible for five deaths in Florence, Ariz. (report 68). According to Randall, nine people ate the poisonous vegetable, while the commission found only five men, in whose cases the evidence was conclusive that they had consumed the product.

In Report 62, it is stated that the woman who escaped illness after eating one olive, vomited immediately after the meal and it is assumed that she eliminated the poison in this manner. This explanation is not absolutely sound. Numerous histories mention initial vomiting notwithstanding which fatal botulism developed in the majority of the cases. The observation of Römer (2) suggesting an uneven distribution of the toxin in ham is supported by the facts detailed in report 86.

CASE MORTALITY RATE.

The number of fatal cases varied greatly in different outbreaks. In 33 observations there were 187 cases with 114 deaths, a case mortality rate of 60.9 per cent. According to Table 10, the case mortality is affected slightly by the foodstuffs responsible for the botulism cases. However, aside from the initial toxicity and the quantity of the poisonous food which has been consumed by the members of the groups or families, the form in which the toxic vegetables or meats have been served, either alone or with other food or reheated or washed before use, influences the mortality rate. Reports 57 and 63 support these general conclusions.

TABLE 10.

Mortality from—	Number of outbreaks.	Number of cases.	Number of deaths.	Percentage.
Commercially pickled and bottled olives	6	45	33	73.3
Commercially canned string beans (2) home-canned string beans (4)	6	24	18	75.0
Commercially canned spinach	4	38	9	23.6
Commercially (2) and home-canned (1) beets	3	33	20	60.0
Home-canned corn	3	12	9	75.0
Home-canned asparagus	1	4	4	100.0
Home-preserved ham and pork products	3	16	14	87.4
Home-preserved (1) and commercially preserved (1) sausage	2	7	4	77.1
Home-prepared cottage cheese	1	3	3	100.0

SEASONAL DISTRIBUTION.

The time of the year had no influence on the number of outbreaks. It is probably mere coincidence that 5 outbreaks occurred in January and also in October. Most of the botulism cases due to home-canned products developed in the fall and winter months for reasons previously discussed.

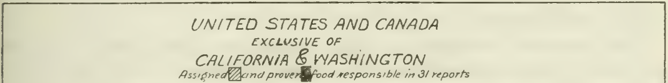


FIG. 7.

FOODSTUFFS RESPONSIBLE FOR THE OUTBREAKS.

The various foodstuffs either assigned as the cause or proven to have been responsible for the botulism in 33 outbreaks are shown in graphic form in figure 7. Plant products caused 72.5 per cent of the cases, while 27.3 per cent of the outbreaks must be attributed to animal products. Commercially bottled olives, commercially canned spinach and home and commercially canned string beans were responsible for one-half of the single or group and family cases. The commission had no means to verify the reports (72, 83, and 87) dealing with tomato catsup, sausages, and smoked ham and mentions the histories merely for the sake of completeness.

The commercially packed products, which were involved in 16 outbreaks, were grown and canned in the following States:

TABLE II.

California	9 outbreaks (6 olives and 3 spinach).
Kansas	1 outbreak (string beans).
Ohio	1 outbreak (beets).
Wisconsin	1 outbreak (sausages).
Unknown	4 outbreaks (1 spinach, 1 beets, 1 string beans and 1 tomato catsup).

The home-canned vegetables or meats which caused 15 outbreaks were grown and preserved in the States of Colorado, Florida, Idaho, Indiana, Iowa, New Jersey, Oregon, New York, and Pennsylvania.

Among the numerous causes which favored the persistence and multiplication of *B. botulinus* in home-canned foods, incomplete sterilization must be considered the most important factor. This statement applies also to the commercially canned ripe olives and spinach. Until quite recently, the processes involved in preserving ripe olives were briefly as follows:

The fruit was permitted to ripen on the trees, care being exercised to start picking before the olives were overripe. After the olives had been gathered by hand, they were transported to the packing plant, where they were sometimes held in dilute brine before they were cured.

In the factory, the olives were first sorted by sizes, then put through repeated washings and conditionings for variable periods depending on the quality and condition of the fruit, the seasonal temperature and the technical details in vogue in the particular plant. In some plants, the olives were kept in agitation during these procedures by means of the ordinary hand paddle, while compressed air jets served the same purpose under more modern practices. The olives were first immersed in clear water in cement or wooden vats for two or three weeks, in order to prepare the skins for the subsequent steps in the process. They were then subjected for from 8 to 12 hours to the action of a solution of caustic soda, 2½ ounces to a gallon of water. After exposure to the air until their coloring was uniform, the olives were again immersed in a solution of caustic soda 1¼ ounces to a gallon of water, for a period of from 8 to 12 hours. The second caustic treatment was to abstract the bitter properties of the fruit and was followed by washing in clear water until all traces of the lye had been removed. The olives were next immersed for one or two days in a brine solution, testing 9 or 10 points on the salometer

(2, 2–2.5 per cent salt) after which they were deposited in bins in the canning room. Women operators then packed them in cans, rejecting at this time all imperfect or soft fruit. The cans were filled with brine testing about 12 points on the salometer (3 per cent salt) at a temperature of 175° to 180° F. and were subjected to an exhaust process of 185° F. before sealing. The fruit was then cooked at 212° to 224° F. for from 12 to 30 minutes depending on the size of the containers and was permitted to cool naturally. No effort was made by any of the canners to control accurately the temperature of the solutions in the various processes.

In a news notice, which was published shortly after the Detroit outbreak, it was mentioned that no heat was used for preservation. This statement is incorrect. All canned and bottled olives have been subjected to heat ever since ripe olives have been canned in California, although until recently the process temperatures employed have been inadequate. Bacteriologic examinations of canned olives by Koser (8) and Rosenberger (9) have revealed the presence of non-spore bearing aerobic bacilli, cocci or members of the colon group even in a small percentage of normal containers; while many living organisms have frequently been obtained from samples which have been abnormal as indicated either by a swelled condition of the container or a bad odor.

In the light of this knowledge, it is truly surprising that only a few containers of bottled ripe olives (14) containing *B. botulinus* or its toxin have been found among the hundreds examined by various laboratories. Experimental studies have indicated that *B. botulinus* grows very poorly in ripe olives and it is quite possible that concomitant multiplication of other bacteria, yeasts or moulds exerts a growth-supporting influence. Variations in the concentration of the brine, prolonged improper preservation of the poorly picked fruit in unsuitable containers before canning may also be contributory factors in the botulism spoilage of olives. From the standpoint of prevention of botulism, however, these factors enact a subordinate rôle to that played by complete sterilization.

The present regulations of the California State Board of Health, which require that all ripe olives canned in brine must be processed at 240° F. for at least 40 minutes and that the brine must be of such concentration as will prevent excessive fermentation, are believed to insure an absolutely safe product.

Little needs to be said with regard to commercially canned spinach. The processing temperature of 212° F. for three hours or 240° F. for 90 minutes for heavily filled No. 10 cans (97 ounces "put in" or 87 ounces net weight) was wholly inadequate, particularly since it is now known that the spinach responsible for 4 outbreaks had been grown on heavily infected soil and had been inadequately blanched before canning. The spinach packers are now submitting their No. 10 cans, with a fill of 82 ounces, and an initial temperature at the center of the can of at least 110° F., to a minimum temperature of 252° F. for at least 90 minutes, and by this process have not only reduced spoilage to a minimum, which they were previously unable to attain, but have in all probability also eliminated *B. botulinus.*

No satisfactory explanation can be offered for the peculiar fact that five outbreaks due to commercially canned olives were recognized in rapid sequence in the course of seven months, although it is known that three were from bottles of the same pack. A similar wave of botulism cases due to spinach suggests that the notoriety given to the first cases prepared the minds of the medical men and health officers so that the poisonous food responsible for the cases was properly identified.

Nothing definite is known concerning the process temperatures employed in canning the commercial beets and string beans assigned as the causative food in reports 68, 74, and 75. When the home-canned vegetables are considered, it is at once noted that string beans and corn are the leading causes of botulism in other States as well as in California and Washington. Asparagus and beets preserved at home are likewise definitely connected with outbreaks.

Apart from the outbreaks due to pork products, the number of cases ascribed to animal foodstuffs is small. The preliminary treatment of the ham is not stated in reports 70 and 80, but it is known that the food was prepared at home. Report 73, dealing with the demonstration of poison in cottage cheese, finds its analogy in report 39 and forcibly calls attention to a food generally considered immune to *B. botulinus* spoilage. It would be of interest to know the character of the milk and the procedures which were employed in making these cottage cheeses. Some of the bacteria commonly concerned in the natural souring of milk, which constitutes the basis for the so-called cottage cheeses, have certain characteristics similar to those of *B. botulinus.* The decomposition products of all these organisms are similar, and without toxicological tests it will be indeed difficult to determine by the odor or by the consistency of the cheese whether or not *B. botulinus* has assisted in the ripening.

PHYSICAL APPEARANCE OF THE INCRIMINATED FOOD.

From the standpoint of safety to the consumer, it is important to know that in the majority of the outbreaks the causative commercially canned food was visibly spoiled. The odor of the olives was invariably declared to be distinctly peculiar, "limburger cheeselike," "musty," or "with a weak putrefactive tinge." In fact, it has been stated that a rancid, offensive odor is characteristic for *B. botulinus* spoilage of olives. Laboratory tests, however, have shown that it is impossible to select toxin containing samples from other spoiled samples without tests on animals. There is unfortunately a widespread lack of acquaintance with the normal odor of ripe olives, and the offensive odor may be reduced to a minimum by washing or icing of the preserved fruit. Any jar or container from which on opening there emanates a peculiar odor should be considered suspicious and the contents discarded. It is recommended that for the destruction of the toxin the contents be thoroughly boiled for at least one hour.

According to reports 63 and 80, canned spinach and beets may show very little spoilage, although they may contain a very potent toxin. The odor may appear normal even on heating, while the physical disintegration may be exceptionally slight. Cloudiness of the brine or liquor may be the only sign of bacterial activity. Indeed, the containers may appear perfectly normal and the absence of a swelled condition of the can may render detection of the spoilage even more difficult. This fact has been recently confirmed by Koser, Edmonson, and Giltner (10) on one can of spinach artificially infected with spores of *B. botulinus*. The home-canned vegetables mentioned in reports 66, 67, 69, 76, 77, 79, and 80 were definitely spoiled and would have been discarded by people who were familiar with the dangers of decomposed food.

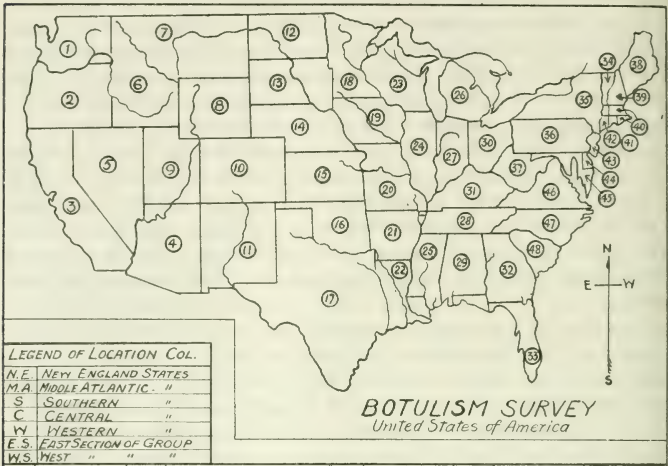
The prevailing view that the poisonous meat must always be "tainted" is not supported by the facts stated in report 70. The ham which caused seven cases had a good appearance and no bad odor.

GEOGRAPHICAL DISTRIBUTION.

A study of figure 8 reveals the following distribution of human botulism in the United States:

	Commercially canned.	Locally grown and home canned.	Unknown.
Arizona	1	0	0
California	12	27	6
Colorado	2	1	0
Florida	0	1	0
Idaho	0	1	0
Illinois	1	0	0
Indiana	1	1	0
Iowa	0	1	0
Maine	0	1	0
Massachusetts	0	2	1
Michigan	3	0	0
Montana	1	0	0
New Jersey	0	1	0
New York	1	3	1
Ohio	1	0	0
Oregon	0	3	0
Pennsylvania	1	1	0
Tennessee	1	0	0
Washington	1	9	1
Wisconsin	1	0	0
Dominion of Canada	1	0	0
Total	29	52	9

It has been pointed out in the introduction that the extensive survey work of the commission is responsible for the data collected in California, which may explain the large number of observations assigned to this State. However, it has been proven that certain States may show an equal frequency of botulism cases provided an effort is made to investigate the distribution more carefully than has generally been the case. In another paper opportunity will be afforded



	NAME		OUTBREAKS						NAME		OUTBREAKS				
STATE NO.		LOCATION IN COUNTRY	HUMAN	ANIMAL	HOME	COMMERCIAL	SOIL %	STATE NO.		LOCATION IN COUNTRY	HUMAN	ANIMAL	HOME	COMMERCIAL	SOIL %
1	WASHINGTON	W.	11	MANY	11	1	80%	25	MISSISSIPPI	S.E.S.				3	INCOMPLETE
2	OREGON	W.	3	"	7		31%	26	MICHIGAN	C.E.S.	3	MANY		1	"
3	CALIFORNIA	W.	45	185	63	19	29%	27	INDIANA	C.E.S.	2	1	1	1	"
4	ARIZONA	W.	1	MANY		1	25%	28	TENNESSEE	S.E.S.	1	MANY			"
5	NEVADA	W.		"			10%	29	ALABAMA	S.E.S.		"		1	"
6	IDAHO	W.	1		1		INCOMPLETE	30	OHIO	C.E.S.	1				15%
7	MONTANA	W.	1	17		1	"	31	KENTUCKY	C.E.S.					
8	WYOMING	W.		MANY			15%	32	GEORGIA	S.E.S.					
9	UTAH	W.		"			25%	33	FLORIDA	S.E.S.	1	MANY	1	1	INCOMPLETE
10	COLORADO	W.	3	"	3	2	33%	34	VERMONT	N.E.					
11	NEW MEXICO	W.					25%	35	NEW YORK	M.A.	4	3	3	1	43%
12	N. DAKOTA	C.W.S.						36	PENNSYLVANIA	M.A.	2	MANY	1	1	14%
13	S. DAKOTA	C.W.S.					8.4%	37	W. VIRGINIA	M.A.		"			INCOMPLETE
14	NEBRASKA	C.W.S.					20%	38	MAINE	N.E.	1		1		"
15	KANSAS	C.W.S.		MANY			10%	39	N. HAMPSHIRE	N.E.					
16	OKLAHOMA	S.W.S.		"			17%	40	MASSACHUSETTS	N.E.	3	2	2		23%
17	TEXAS	S.W.S.		"			5%	41	RHODE ISLAND	N.E.					
18	MINNESOTA	C.W.S.		"			28%	42	CONNECTICUT	N.E.					
19	IOWA	C.W.S.						43	NEW JERSEY	M.A.	1	MANY	1		31%
20	MISSOURI	C.W.S.		MANY			46%	44	DELAWARE	M.A.					
21	ARKANSAS	S.W.S.		"			19%	45	MARYLAND	M.A.		2	2		54%
22	LOUISIANA	S.W.S.		"			10%	46	VIRGINIA	M.A.		MANY			INCOMPLETE
23	WISCONSIN	C.E.S.	1	"		1	10%	47	N. CAROLINA	S.E.S.					10%
24	ILLINOIS	C.E.S.	1	"		1	26%	48	S. CAROLINA	S.E.S.					INCOMPLETE

FIG. 8.

to consider the relationship of human botulism to the distribution of *B. botulinus* spores in the soil of certain States. Studies thus far completed indicate that several areas in the United States may harbor comparatively few spores and consequently botulism is either absent or is introduced with food products from territories in which the organism is common.

Part VI.

BOTULISM IN DOMESTIC ANIMALS.

In 1917 Dickson (11) and Buckley and Shippen (12) in two separate publications called attention to the striking similarity of botulism in man with "limberneck" in chickens and forage poisoning in horses. These papers were soon followed by the reports of Graham and his associates (13) dealing with some forms of intoxication in horses and cattle, which were diagnosed as botulism. Aside from these publications the medical literature records also observations of accidental poisoning in hogs, cats, and dogs accidentally contracted by eating spoiled discarded food which had been responsible for human botulism. A careful review of the various publications justifies the following conclusions: The toxin of *B. botulinus* is frequently responsible for the death of barnyard fowls in rural and urban poultry flocks in North America. On the other hand the etiologic relationship of this toxicogenic bacterium to forage poisoning in horses and cattle is based in the majority of instances on indirect evidence. *B. botulinus* is so widely distributed in nature that the mere isolation of this organism from the suspected feed or intestinal tract or spleen of a dead animal is insufficient to support the diagnosis of botulism.

Inasmuch as botulism in poultry accompanied a number of human outbreaks, the commission has attempted to collect and to analyze the available information. Moreover botulism of domestic animals probably assumes in certain localities an important economic aspect, and a critical summary offers an opportunity to discuss some of the problems in animal pathology which deserve careful experimental investigation.

A. BOTULISM IN BARNYARD FOWLS.

Through the observations of Dickson it has been established that a toxemic syndrome is produced in poultry following the accidental or experimental consumption of spoiled food containing the toxin of *B. botulinus*. The affected chickens, turkeys, or ducks exhibit the following symptoms: The birds are inactive and dull, refuse to eat, remain quiet in one place with the feathers ruffled. As a rule the movements of the nictitating membrane are sluggish and sometimes there is a marked constipation, but the general picture of extreme weakness governs all the symptoms; the wings droop and the beak or side of the head rests upon the floor of the cage or barnyard. This latter striking feature of the disease is popularly known as "limberneck." During this stage of the malady the chickens are still able to lay eggs. The clinical manifestations are exceedingly characteristic and easily diagnosed. Although it is sometimes impossible to determine with absolute certainty the cause of sporadic limberneck, the field observations to be presented definitely show that botulism in fowls differs clinically and etiologically from "leg weakness" and "wry neck," two dietary deficiency diseases commonly observed in poultry yards.

In order to avoid further confusion this intoxication of barnyard birds should be frankly designated as botulism instead of limberneck. Until epidemiological and bacteriological studies have proven the contention of Graham and Schwarze (14) to be correct, the prefix "avian or type A" botulism appears unnecessary, misleading, and should be discontinued.

The experiments of Wilkins and Dutcher (15) indicate that limberneck is a symptom rather than a disease resulting from the partial or complete loss of control of the muscles of the neck and probably follows not infrequently the ingestion of the larvæ of *Lucilia caesar.* Several years ago Saunders (16) concluded from an elaborate series of tests that the larvæ of the green flies (*Lucilia caesar*) after being contaminated from carrion flesh play an important rôle in the development of limberneck in poultry. The nature of the poison present in the fly larvæ has not been determined. Some of the statements by Saunders and some experiments on guinea pigs reported by Wilkins and Dutcher suggest that the larvæ may contain the toxin of *B. botulinus,* which originated in the carrion.

Number of outbreaks and mortality rate.—The reports dealing with outbreaks of botulism in poultry which have been collected and investigated by the commission are summarized in Table 12; a number of observations are reported for the first time.

TABLE 12.

No.	Food responsible.	Species of bird and number of deaths.	Location.	Date (year and month).	B. botulinus isolated.	Type.	Coincidence with human cases.	Remarks and recorded by –
1	Home-canned string beans	Chickens	San Jose, Calif.	1910				E. C. Dickson.
2	do	25 chickens, 1 turkey	Madera, Calif.	1915				Do.
3	do	130 chickens, 30 turkeys	Santa Ysabel, Calif.	1915				Do.
4	do	80 chickens, 6 turkeys	Escondido, Calif.	1915				Do.
5	do	25 chickens	do	1915				Do.
6	do	8 chickens	San Jose, Calif.	1916, Jan.	Crop and gizzard of 3 chickens; 6 examined.	A	1 case	Do.
7	do	25 chickens	San Pasquale, Calif.	1916, July			do	Do.
8	do	52 chickens	Berkeley, Calif.	1917				Do.
9	do	12 chickens	do	1918, Feb.	3 jars of beans and gizzards of 2 chickens.	B		Do.
10	do	72 of 81 chickens	Raymond, Calif.	1918, Jan.				Do.
11	do	25 chickens	Sacramento, Calif.	1918, June	Jar of beans and crop of chicken	B		G. S. Burke.
12	do	15 chickens	Santa Maria, Calif.	1918, Oct.	Jar of beans	A		E. C. Dickson.
13	do	Several	Sacramento, Calif.	1919				Commission.
14	do	do	Los Angeles, Calif.	1919, Feb.				Do.
15	do	25 chickens	Oroville, Calif.	1919, Mar.				Do.
16	do	634 chickens	Saratoga, Calif.	1920, Jan.	Caeca and colon of 1 chicken; A toxin in crop.	A		Commission and Dr. Hart, University of California.
17	do	23 chickens	Los Angeles, Calif.	1920, July	Intestinal contents and wall	A		2 jars cold pack; second jar leaking positive in 3 days.
18	do	2 chickens	do	1920, Apr.			1 case	Commission.
19	do	25 chickens	do	1920, Apr.	Crop, gizzard, and intestines of chicken; no toxin in crop.	A		Los Angeles Health Department (Mr. Miller).
20	do	30 chickens	Newmann, Calif.	1920, Aug.				Commission.
21	do	4 of 10 chickens; ducks not affected.	Los Angeles, Calif.	1921, Jan.				Do.
22	do	22 chickens	Mayfield, Calif.	1921				Do.
23	do	25 chickens	Lyons, Colo.	1920, Nov.	Dried crop contents cultured gave a weak toxin; enriched type A.			Doctor Newsom, Fort Collins.
24	do	Several	Decatur, Ind.	1918, Feb.	Jar of beans	B?	7 cases	McCaskey, Amer. Journ. Med. Sci., 1919, *158*, 57.
25	do	do	Klamath Falls, Oreg.	1918, Oct.				E. C. Dickson.
26	do	30 chickens	do	1920, Feb.	Chicken crop and gizzard; A toxin in crop.	A		Commission.
27	do	Several	Newark, N. J.	1918			3 cases	Investigated by de Bord, Journ. Amer. Med. Assn., 1919, *73*, 912.
28	do	24 chickens	Reno, Nev.	1920, Jan.				Commission.
29	do	39 chickens	Ontario, Oreg.	1917, Dec.			1 case	E. C. Dickson.

30	do.	20 chickens	Yelm, Wash	1921, Feb.	Jar of beans	A	do.	Commission.
31	Home-canned corn	25 to 30 chickens	Corning, Calif	1917, Jan.			do.	Do.
32	do.	25 chickens	Hanford, Calif	1917				E. C. Dickson.
33	do.	58 of 66 chickens	San Jacinto, Calif	1917, July				Do.
34	do.	25 chickens	Visalia, Calif	1917				Commission.
35	do.	35 chickens	Calistoga, Calif	1918, Jan.				Do.
36	Commercially canned corn.	10 chickens experimentally	San Rafael, Calif	1918, May.	Crops and gizzards of 2 chickens; negative.		1 case	Experimentally produced by health officer, Journ. Amer. Med. Assn., 1919, 74, 1079.
37	Home-canned corn	25 chickens	Santa Rosa, Calif	1919, Oct.				Commission.
38	do.	50 chickens	Escondido, Calif	1920, Apr.				Do.
39	do.	35 chickens	Biggs, Calif	1920, Aug.	Chicken gizzard 6 weeks in soil	A	1 case	Do.
40	Spoiled, moldy corn	30 chickens	Los Angeles, Calif	1921, Jan.				Dr. Carr, Los Angeles.
41	Home-canned corn	25 chickens	Fort Collins, Colo	1920	Dried crop contents cultured gave weak toxin.			Dr. Newsom, Fort Collins.
42	do.	Several	Washington, D. C.	1920, Oct.	Crop of chicken	B		Report of Bureau of Animal Industry, Oct. 13, 1920.
43	do.	50 chickens	Hillsboro, Oreg	1915, Oct.	Contents of gizzard of chicken advanced decomposition.	A	1 case	E. C. Dickson.
44	do.	30 chickens	Yakima, Wash	1916, Jan.			do.	Commission.
45	do.	50 chickens	do.	1916, June.				Do.
46	do.	25 chickens	do.	1917, Nov.			1 case	Do.
47	do.	30 chickens	do.	1918, Dec.			do.	Do.
48	do.	25 chickens	do.	1919, June.				Do.
49	do.	30 chickens	do.	1920, July.				2 horses; commission.
50	do.	50 chickens	do.	1920, Dec.	Jar of corn	A	1 case	Commission.
51	do.	25 to 50 chickens	do.	1921, Apr.	B. toxin crop; B. botulinus not isolated.	B		Commission; chicken buried 2 weeks.
52	Home-canned asparagus	30 chickens	Oroville, Calif	1919, Mar.				Commission.
53	do.	50 chickens	Menlo Park, Calif	1920, Aug.	Decomposed chicken crop; no toxin in crop.	A		Do.
54	do.	30 chickens	San Jose, Calif	1920, Nov.				Do.
55	do.	Several	Seattle, Wash	1917	Contents of crops and gizzards of 7 chickens; 10 examined.	A	1 case	E. C. Dickson.
56	do.	12 chickens	Yakima, Wash	1910			1 case	Commission.
57	Home-canned apricots	Several	Fallbrook, Calif	1915, Mar.			5 cases	1 dog; E. C. Dickson.
58	do.	25 chickens, 1 turkey	Madera, Calif	1918, Jan.	Gizzard of 1 chicken	A	8 cases	E. C. Dickson.
59	Bean hay	150 chickens	Burbank, Calif	1918, May.				Dr. Hurt, Los Angeles.
60	do.	40 to 50 chickens	Colusa, Calif	1920, Mar.	Bean plant	A		Also horses; commission.
61	Home-canned peas	50 chickens	Yakima, Wash	1920, June.				Commission.
62	do.	30 chickens	do.	1921, Jan.				Do.
63	do.	25 chickens	El Cerrito, Wash	1921, July.				Do.
64	Home-canned spinach	15 chickens	Toppenish, Wash	1921, Apr.			2 cases	Do.
65	do.	10 chickens	McKenna, Wash	1919, May.			3 cases	Do.
66	do.	Several	Battle Creek, Mich	1921, Jan.				Reported to commission by Geagley, Mich.
67	Home-canned tomatoes	26 chickens	Los Angeles, Calif	1920, July.				Commission.
68	Commercially canned tomato paste.	11 ducks fed (2 dead)	do.	1916, Aug.				Reported by A. Schneider to commission; seized goods.
69	Moldy bread	20 chickens	do.	1920, Nov.				Commission.

No.	Food responsible.	Species of bird and number of deaths.	Location.	Date (year and month).	B. botulinus isolated.	Type.	Coincidence with human cases.	Remarks and recorded by—
70	Rotten potato peelings	10 chickens	Los Angeles, Calif.	1920, Feb.	Crops of chickens negative according to a report from a laboratory.			Commission.
71 86	16 observations due to raw buried potatoes.	About 600 chickens	Yakima, Wash.	1915–1921				Do.
87 88	2 observations, moldy potatoes.	25 chickens	Reno, Nev.	1920				Do.
89	Spoiled sour milk	20 chickens	do	1921, July.	Crop and gizzard of 1 chicken negative.			Dr. Vawter, Reno, Nev.
90	Commercially canned tuna	12 chickens	Los Angeles, Calif.	1920, Feb.			1 case	Commission.
91	Home-canned salmon	10 chickens	Redding, Calif.	1920, Nov.				Do.
92	Pickled fish	75 chickens	San Jacinto, Calif.	1921, July.	Crops, gizzards, and intestines of 2 birds; A type toxin in crop of 1 chicken.	A	1 case	California State Board of Health and Bureau of Chemistry, western division.
93	Home-canned chicken	25 chickens	Delhi, Calif.	1921, June.	Jar of chicken	A		Commission.
94	Spoiled canned goods (unknown variety and method).	10 chickens	Reno, Nev.	1920				Do.
95	Garbage	Several	Illinois	1920	Garbage and intestines of chickens.	A		Graham and Schwarz, Journ. Infect. Dis., 1921, *28*, 317.
96	Unknown	100 chickens	Hornbrook, Calif.					Commission.
97	do	20 chickens	Los Angeles, Calif.					Do.
98	do	15 chickens	Elk Grove, Calif.					Do.
99	do	20 chickens	Colusa, Calif.					Do.
100	do	15 to 30 chickens	Oakdale, Calif.					Do.
101	(Clams?)	10 chickens	San Jose, Calif.					Do.
102	(Chicken feed?)	35 chickens	Stockton, Calif.					Do.
103	(Chicken feed?)	25 chickens	do					Do.

ADDITIONS SINCE JULY 31, 1921.

No.	Food responsible.	Species of bird and number of deaths.	Location.	Date (year and month).	B. botulinus isolated.	Type.	Coincidence with human cases.	Remarks and recorded by—
104	Home-canned corn	27 chickens	El Segundo, Calif.	1921, Aug.				Commission.
105	do	9 chickens	Yakima, Wash.	1921, Oct.	Crop of 1 chicken contained type A toxin and B. botulinus isolated.	A	1 case	Health officer; Yakima and commission.
106	Home-canned asparagus	23 chickens	Walla Walla, Wash.	1921, Oct.	3 jars of same pack			Bureau of Chemistry, western division.

In reviewing the data presented in Table 12 certain facts can be determined. Since 1910, 103 reports dealing with botulism in poultry have been collected, although there is no doubt that this figure is incomplete since no search for them has been made in the majority of States of the Union. Moreover it is the belief of the commission that even the data available for the State of California are incomplete because as a rule the occurrence of botulism in poultry yards has been accidentally recorded in the course of an inquiry into an outbreak of human food poisoning. It is not unlikely that only extensive outbreaks attracted attention, while numerous sporadic cases have not been recognized or are only known to a small group of people. The losses sustained by the owners from this intoxication are sometimes considerable and the problem of distribution and frequency of botulism in barnyards unquestionably deserves more attention.

In Table 13, 79 outbreaks of botulism in fowls are arranged according to years. The frequent careless disposal of spoiled home canned food and the recognition of "limberneck" as true botulism probably explain the higher incidence of the last 4 to 5 years.

Table 13.—*Annual incidence of botulism in barnyard fowls.*

1910	2	1918	12
1911–13	0	1919	7
1914	1	1920	26
1915	5	1921 (until June 30)	11
1916	5		
1917	8	Total	79

It is usually impossible to determine accurately the number of chickens which died following the eating of toxic food, but in some outbreaks (report 16) the mortality has been very high. The 3,500 chickens, which died from botulism in the 103 outbreaks represent a direct financial loss of approximately $7,000. Considering the indirect losses (value of eggs and carcasses) it will be appreciated that this intoxication has some economic importance, particularly in the Yakima Valley.

Nothing definite is known with regard to the intoxication and mortality rate. As a rule all the birds which consumed the poisonous food showed symptoms and succumbed in from 48 to 96 hours, although death may not occur until after one to three weeks. Occasionally several very large birds showing marked symptoms were seen to recover spontaneously after a prolonged illness of several weeks. Some reports state that only few birds exhibited symptoms or died after eating the food; in these instances the housewives remembered that they had warmed or cooked the spoiled vegetables for a short period before throwing it to the chickens. The mixing of toxin containing spoiled vegetables with garbage may lead to disastrous outbreaks as is well illustrated by report 16 and the recent publication of Hart (18).

The majority of reports mention chickens as the only birds affected. In three outbreaks fatalities among turkeys have been reported, while in one observation (report 21) it is stated that several ducks which consumed the spoiled beans remained perfectly well. According to Dickson (17) and Hart (18) wild birds (wild canary and California linnet) which may have access to the feeding troughs have been seen to develop botulism.

Unfortunately many of the records fail to state the months in which the outbreaks occurred and it is impossible to establish anything definite with regard to the seasonal distribution of the intoxication in poultry. From previous statements, one may assume that most of the outbreaks occur during the winter months when spoiled canned or preserved food is carelessly discarded. This is not entirely the case. At least in California a number of outbreaks was observed

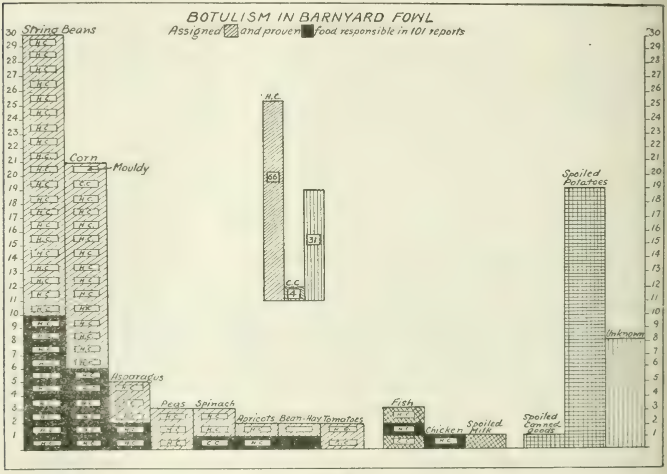


FIG 9.

during the months of June, July, and August, probably because home-canned vegetables preserved in the early summer undergo fermentation during the hotter months and the housewife immediately discards the spoiled food, occasionally using it as chicken feed.

Foodstuffs responsible.—A casual perusal of the data presented in figure 9 supports again the contention that plant products are the foodstuffs primarily responsible for botulism in chickens, as in man. Aside from the well-known vegetables like string beans, corn, asparagus, spinach, and peas, other plant products are epidemiologically suspected as sources and vehicles for the poison. Numerous observations in Yakima Valley definitely incriminate raw buried or moldy potatoes and potato peelings. It is to be regretted that the diagnosis of botulism thus far depends on a study of symptoms. However it has been possible to demonstrate *B. botulinus* in some samples collected from several potato pits in the vicinity of these outbreaks. Potato poisoning of animals has been attributed to bacterial decomposition with proteus bacilli and in some cases to a poisonous alkaloid, "solanin," said to be present in excessive amounts in diseased and in sprouting potatoes. The latter conception of poisoning due to potatoes is however doubted by the majority of investigators. Similarly inconclusive are the statements that chickens developed botulism following the consumption of moldy bread or bean hay, even though laboratory tests supported the view of the careful observers who reported these cases. Both plant products are suitable for the growth of *B. botulinus* provided sufficient moisture is present. However it is not unlikely that the birds also had access to other spoiled poisonous vegetables. This suspicion has been proven at least in one instance (report 19).

A dead chicken was sent to the laboratory with the statement that a number of the same flock had died with the symptoms of "limberneck." The owner was definite in his statements that the birds were only fed commercial chicken feed. On autopsy, however, a fragment of a string bean was found in the crop and *B. botulinus* was isolated from this specimen. It was never determined where the chickens found the remnants of the toxic food.

In the light of this observation the members of the commission desire to state that they have recorded in Table 12 the causative food products mentioned in the reports submitted by the owner or the veterinarians. There seems to be no doubt that the birds died from limberneck, but it is possible that the causative food had not been determined accurately and that the poison responsible for the intoxication was generated by other bacteria than *B. botulinus*. Invariably an effort has been made to prove the diagnosis by toxicological or bacteriological tests, but in the majority of instances the carcasses had been buried and the demonstration of *B. botulinus* in the decomposed cadavers was of doubtful value.

One-half of the botulism outbreaks in chickens were presumably caused by home-canned string beans and corn. Experimentally 10 chickens were poisoned by commercially canned corn. In a few instances home-canned asparagus, peas, apricots, and spinach were considered the toxin-carrying food. It is noteworthy that spoiled tomatoes, even in one instance commercially canned tomato paste, produced botulism in chickens. According to report 68, 11 ducks were fed experimentally with tomato paste (8 cc. each); all developed clinical botulism and two succumbed. Furthermore, the observations presented in reports 90 and 93 definitely suggest the possibility that both home-canned salmon or fried chicken may undergo spoilage due to *B. botulinus.* The presence of the bacillus in the spoiled chicken was demonstrated by bacteriologic tests. Spoiled sour milk is unquestionably an excellent medium for *B. botulinus* and it is surprising that only one single chicken outbreak epidemiologically attributed to it has thus far been recorded. The long list of outbreaks of botulism in barnyard birds mainly caused by home preserved vegetables, fruits, and fish conclusively proves. the contention of the commission, that the methods of preservation can not be considered safe. According to the available records the food products were always spoiled, but the physical disintegration was frequently slight and the vegetables were therefore used as chicken feed. Attention has already been called to the difficulty encountered in recognizing spoilage of string beans, and this peculiar behavior explains in part the high percentage of outbreaks definitely attributed to this vegetable. Probably all of these losses could have been avoided had the housewives followed the recommendations repeatedly made that spoiled vegetables or fruits should not be discarded without previous boiling for at least 30 to 45 minutes.

Certain observations dealing with the behavior of the toxin of *B. botulinus* deserve additional consideration, and are therefore described below;

It is stated in report 17 that Mrs. J. W. Bl. canned on July 23, 1920, two gallon jars of string beans by the cold-pack method. One jar was cooked for one hour and served at the noon meal on July 25. On July 26 on account of a leaking rubber ring the contents of the second jar were discarded and thrown in the chicken yard. The whole flock of 23 chickens showed symptoms of botulism on July 27, and all succumbed in from two to five days. *B. botulinus*, type A, was isolated from the intestinal content. This observation definitely indicates that in the course of three warm summer days a strong *B. botulinus* toxin can be elaborated in a jar of home-canned string beans.

In report 23 it is noted that Mrs. C. B. H. found in the cellar a jar of spoiled string beans. The container was placed outdoors and forgotten. On account of the cold weather the contents froze and broke the jar. The débris was thrown into the chicken yard and caused the death of 25 chickens. This observation supports the laboratory studies which have shown that the toxin of *B. botulinus* is not materially altered by freezing.

Bacteriological or toxicological diagnosis. –It is to be regretted that the diagnosis of botulism was only supported by a bacteriological examination in 23 instances. In 8 outbreaks it was possible to test the causative food by toxicological or cultural methods. In six jars of spoiled vegetables the toxin of *B. botulinus*, types A and B, was demonstrated, while in the remaining two instances the organism was isolated with the aid of enrichment cultures.

Cadavers of chickens were examined in 20 outbreaks. In three chickens obtained from 4 different outbreaks (reports 16, 26, 51, and 92) the presence of *B. botulinus* toxin was demonstrated by the inoculation of guinea pigs with filtered saline extracts of the crop content. The toxin type was also established with the same material in three instances (reports 26, 51, and 92). In this connection it should be mentioned that the dead chicken of 1 outbreak (report 26) had been buried for three weeks in frozen soil. The cadaver was still frozen and in an excellent state of preservation when received at the laboratory. The filtered extract of the crop content killed a 250-gram guinea pig within 24 hours. No toxin could be extracted from the crop contents of two partially decomposed carcasses of chickens obtained from the outbreaks mentioned in reports 19 and 53. The isolation of *B. botulinus* from the crop, gizzard, or intestines of dead chickens was successful in 14 instances. It is not unlikely that the failures mentioned in four reports (reports 36, 51, 70, and 89) must be ascribed to the cultural methods employed. However, it is well known, that the isolation of *B. botulinus* can not always be accomplished from all the dead chickens of one and the same outbreak (reports 6 and 55). The following observation is of some interest in this connection: A bird, which died on the sixth day after the consumption of the poisonous food, harbored the spores of *B. botulinus* only in the cæca, while the crop, gizzard, jejunum, colon, spleen, and liver gave negative cultures. Until quite recently the demonstration of *B. botulinus* in the intestinal tract of chickens, even many days after death and in badly decomposed cadavers was considered conclusive evidence to support the clinical diagnosis of botulism. This conception is now believed to be incorrect for the following reasons:

The spores of *B. botulinus* are widely distributed in nature. They are found in the soil, on vegetables, in chicken feed, etc., and they are probably present with other anaërobes during the decomposition of protein material and in carrion. The methods employed to demonstrate the presence of *B. botulinus* are very delicate; a few spores of *B. botulinus* can germinate in the enrichment mediums generally employed and their progenies can elaborate sufficient poison to give a positive toxin test on guinea pigs and mice. Numerous observations to be reported elsewhere suggest that extreme caution should be exercised in the interpretation of positive cultures obtained from tissues or organs of birds or animals that have been exposed to contact with the soil and its products. The finding of *B. botulinus* in garbage or chicken feed lacks etiologic significance, provided the existence of its toxin in the apparently wholesome or obviously spoiled food can not be simultaneously demonstrated. It is, therefore, frankly admitted that the bacteriological findings on chicken cadavers summarized in Table 12 have very little diagnostic value. Future studies should attempt to establish the existence of the specific toxin in the suspected food or in the crop content of the dead birds. How readily any mysterious disease of birds can be diagnosed as botulism is well illustrated by the following observation:

Two wild ducks were received by the laboratory from a town in Missouri. In the accompanying history it was stated that a large number of the flock had died in the course of from one to three weeks. An examination of the badly decomposed carcasses revealed the lesions of coccidiosis; a few oocysts were demonstrated microscopically. The composite sample of the intestines of one bird, however, gave a culture of *B. botulinus*, type B.

Is B. botulinus, type B, responsible for botulism in poultry?—Graham and Schwarze (17) concluded from some laboratory studies, that *B. botulinus*, type A, administered via the digestive tract is fatal to mature chickens, while type B is nontoxic. Reliable investigations have shown, that at least in four outbreaks (reports 9, 11, 42, and 51) *B. botulinus*, type B, was found in the causative food or the crop of the chickens. The presence of a type B toxin in the crop content of one chicken (in report 51) refutes the contentions of Graham and Schwarze that outbreaks of chicken botulism are always due to type A toxin. Hart and Hayes (19), members of the commission, and workers of the Bureau of Animal Industry have repeatedly noted that chickens are very resistant to a type B intoxication via the digestive tract. It is, however, not unlikely that the differences in susceptibility of poultry to the two types of *B. botulinus* is merely a quantitative instead of a qualitative one. It is suspected at least from an epidemiological standpoint that both toxin types can cause fatal intoxications in chickens but future studies should seriously consider the possible occurrence of a mixture of types in the causative food and in the intestinal canal of chickens, which have been buried for several days before being examined. Quantitative toxicological tests with the causative food or the saline extracts of the crop content of birds, which are not decomposed, will alone settle the uncertainty created by the publication of Graham and Schwarze.

Geographical distribution.—The available reports place the outbreaks of botulism in chickens in the following States:

[No systematic studies made outside of California.]

California	56	New Jersey	1
Colorado	2	Nevada	5
District of Columbia	1	Oregon	4
Illinois	1	Washington	31
Indiana	1		
Michigan	1	Total	103

The food responsible for human botulism caused in 22 instances the same intoxication in barnyard birds and other animals. This connection between human and animal botulism is therefore invaluable from an epidemiological standpoint and has frequently assisted the medical man to diagnose the nature of the food poison.

B. BOTULISM IN HORSES.

In the course of the epidemiological investigations discussed in this report the commission had occasion repeatedly to record and to study outbreaks of so-called "forage poisoning" of horses. Inasmuch as some of the fatalities among equines were due to foodstuffs which contained the toxin of *B. botulinus* it is considered advisable to briefly analyze the available information and to review the present knowledge on forage poisoning.

In 1900, L. Pearson (20) investigated in Pennsylvania an outbreak of poisoning among horses due to eating moldy silage. The disease was confined to seven horses in a stable; five died after two to four days and two recovered. No autopsies were made. Two horses fed experimentally on the same lot of silage also died after showing tremors, paralysis of the pharynx, and general paralysis (paresis ?), but no brain symptoms were noted. Pearson called attention to the errors in diagnosing diseases of this class as cerebrospinal meningitis because there is no evidence of an inflammation in the meninges of the brain or spinal cord. Since the disease resembled closely "sausage poisoning" in man and since it was evident from his observations that the toxic principles resided in the food, he suggested the name "forage poisoning." Unfortunately this term has not been restricted to sickness in animals due to eating decomposed, moldy, or fermented food as ensilage, but certain diseases of horses (enzootic encephalitis or Borna's disease) which are characterized by cerebral instead of peripheral nerve symptoms have also been diagnosed as forage poisoning.

According to the recent studies of Kraus, Kantor, and Quiroga (21) in Argentina one form of encephalitis is probably caused by a specific diplococcus. On the other hand the review of Udall (22) dealing with the extensive outbreaks of "enzootic encephalitis," reported in 1912 from Kansas, Nebraska, and New Jersey fails to mention the finding of diplococci. The anatomical lesions found and described by McCarthy and Ravenel (23) in an outbreak of forage poisoning are again unlike those described for enzootic encephalitis. From the various reports it seems possible that at least three or four specific diseases of horses, which are etiologically different produce symptoms of similar character. In fact equine botulism bears from a differential diagnostic standpoint the same relation to enzootic encephalitis and allied infections of the central nervous system of horses as human botulism bears to "epidemic encephalitis," cerebral lues, etc., in man.

There occurs however, as already stated, a malady in horses in which it has been repeatedly possible to establish an inseparable relationship between the feed containing a poisonous substance and the intoxication following its ingestion. In 1917 Graham and his associates (16) and Buckley and Shippen (15) suggested that in a number of outbreaks the toxin of *B. botulinus* may represent the poisonus substances in the feed. These workers have definitely shown that the symptons and post mortem findings exhibited by some experimentally intoxicated horses and donkeys resemble those generally noted in true forage poisoning, namely stupor, drooping of the head, marked weakness of the legs and muscles in general, incoordination of the movements, staggering gait, paralysis of the pharynx, paretic, oedematous tongue, salivation,[6] subnormal, or normal temperature, normal or rapid pulse, accelerated and shallow respiration, inability to swallow, and obstinate constipation. The following lesions are found at autopsy: General congestion of the abdominal viscera, marked generalized or patchy hyperaemia of the lungs; petechiae on the serous membranes of the heart and injection of the meninges. Microscopic examinations of the nervous tissues have not been reported. Although the practicing veterinarian is frequently not in a position to observe all of the above mentioned symptoms and lesions, it should however be born in mind that they are sufficiently distinct to make a differential diagnosis between an infections disease involving the nervous system and this intoxication due to a poisonous substance in the feed. On epidemiological grounds the action of poisonous plants can usually be excluded.

Since Graham and his associates championed the etiological rôle of *B. botulinus* in forage poisoning there has developed a tendency to diagnose any mysterious disease of horses, cattle or hogs as botulism. This state of affairs is most unfortunate because the experimental evidence thus far produced is inconclusive. The mere demonstrations of *B. botulinus* enrichment cultures from the suspected feed, a procedure which even in the hand of the experienced laboratory worker is not always successful, is seriously invalidated by the recently established and yet unpublished data indicating the ubiquitous distribution of this bacterium in the soil and vegetation of almost every State in the Union. It should also be stated that *B. botulinus* has been isolated from perfectly sound and wholesome horse and cattle feed. Furthermore the toxin has been readily produced by inoculating and incubating at 27° C. numerous aerobic nonsterile hay, straw, and corn infusions with the spores of *B. botulinus*. In order to prove the etiologic significance of *B. botulinus* in forage-poisoning cases, the presence of the toxin in the feed, its resistance to light, desiccation, and heat under the conditions prevailing in nature should be established by well planned experiments. Some observations of Hart and Hayes (22) make it probable however, that only horses can be used for this purpose. Determination of the relative susceptibility of horses to the ingestion of *B. botulinus* toxin compared with that of guinea pigs, rabbits, mice, cattle, etc., are some of the problems which deserve immediate investigation.

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[6] There is some question as to whether there is true salivation or whether an apparent increase in salivary secretion exists because the animal is unable to swallow.

In applying these critical considerations to the accounts of forage poisoning in horses and mules either published or collected by the commission, it must be concluded that the toxin of *B. botulinus* was probably the cause of equine botulism in the following four instances:

(1) Five burros died after eating the garbage which contained the remnants of commercially canned string beans or spinach which had been responsible for an outbreak of typical botulism in human beings (see report 74) in Colorado. This occurrence was reported by Curfmann in 1917 (24).

(2) Forty heads of mules were being fed ensilage on a farm in Carroll County, Ky. A number of mules became ill, manifesting symptoms of botulism. Two horses which had been injected with antitoxin and fed on the suspected poisonous ensilage remained well, while one control horse showed symptoms of forage poisoning and succumbed. Reported by Graham, Brueckner, and Pontius in July, 1917 (13).

(3) Nine horses and mules were fed corn ensilage at Ottawa, Ill., 1916. Six of the animals died. Two feeding experiments were conducted on 18 horses; 14 animals received prophylactic injections of botulism antitoxin while four horses served as controls. Only the treated animals except one which died from an intercurrent peritonitis survived. Reported by Graham and Brueckner, January, 1919 (13), and Rusk and Grindley, 1918 (25).

(4) Two horses together with 30 chickens consumed the remnants of a jar of spoiled home-canned corn which had been thrown into a yard at Yakima, July, 1920. The animals developed botulism and succumbed. Investigated by the commission.

The above histories indicate that horses, following the ingestion of preserved or fermented foods, which are known to contain the toxin of *B. botulinus*, may develop a symptom complex typical for forage poisoning. Improperly packed silos undergoing abnormal fermentation undoubtedly offer excellent opportunities for the ubiquitous *B. botulinus* to generate its toxin. It appears desirable to demand, that in the future problems of so important a nature be carefully investigated and the factors which influence the development of bacterial toxins in the ensilage be studied experimentally in the States which use the silo extensively. In this connection it can be stated, that moldy silage of an alkaline reaction is probably more suitable for *B. botulinus* than the same food, which had undergone vigorous lactic acid fermentation. Equine botulism due to silage and home canned products can be more readily controlled by methods other than the use of expensive serums.

In addition to the four indirectly proven outbreaks, a large number of instances of forage poisoning in horses due to moldy hay, straw, corn, potatoes, etc., are being considered in California and in several other States as identical with botulism. Inasmuch as the accuracy of the diagnosis was considered exceedingly doubtful in the majority of the cases reported, the commission has made an attempt to collect further data and opinions from authoritative observers, by sending a circular letter regarding forage poisoning to all the State veterinarians in the Union. The replies were mostly noncommittal and inconclusive, but in order to stimulate further research the data are arranged according to States and are presented in subsequent paragraphs.

(1) *Alabama.*—Reports frequent single and group cases in spring and early summer.

(2) *Arizona.*—Many outbreaks; certain weeds (filiria) and grasses are suspected; repeated examination of such material by the laboratory of the commission failed to demonstrate *B. botulinus.*

(3) *Arkansas.*—No report available.

(4) *California.*—The incomplete reports received by the commission for the years 1911–1920, mention 78 outbreaks of forage poisoning with a total mortality of 123 horses. Twenty-three of the outbreaks occurred in the northern, 20 in the central, and 32 in the southern section of the State. In 35 instances the following feeds were suspected:

Moldy hay	20	Moldy beets	1
Moldy corn	4	Alfalfa	1
Moldy barley hay	4	Straw	1
Grain hay spoiled	2		
Moldy potatoes	2	Total	35

In 42 reports no information relative to the feed was given; on some cards filled out by veterinarians it was mentioned that the disease occurred among pasture animals. In this connection the following observations deserve a detailed description.

(*a*) Colusa: March, 1920, a horse which had been fed bean hay and straw exhibited a staggering gait and disturbed vision, nervous, aggressive attitude, muscular weakness, rapid shallow respiration and coma. The temperature was 100° to 101° F. and later 98° F. The horse died on the third day after the onset of the symptoms.

B. botulinus, type A, was isolated from the dry and clean bean plant. The same organism was also found in several soil samples; 40 to 50 chickens eating the same material died with symptoms of botulism. (See chicken botulism report 60.)

(*b*) Whittier: June, 1920, one horse of three kept on the same premises showed difficulties in swallowing, trembling, paralysis of the tongue, symptoms of colic, general muscular weakness and later coma. The autopsy revealed no gross lesions, but an increased amount of cerebro spinal fluid. Cultures from the spleen and liver were negative. A 10-gram and a 5-gram sample of the intestinal content (colon) contained *B. botulinus*, type A. Three other specimens from other sections of the intestinal tract gave negative cultures. A sample of the hay was nontoxic for guinea pigs, but gave a culture of *B. botulinus*, type B. The other two horses received 50 cc. each of *B. botulinus* antitoxin (University of California), types A and B; they later ingested the same hay without developing forage poisoning; unfortunately nonimmunized horses were not available as controls.

(*c*) Santa Maria: February, 1920, the moldy barley hay which is suspected of having caused forage poisoning in one horse contained *B. botulinus*, type B.

(*d*) Fresno County, near Kings River: November, 1920, the bacteriological examination of the organs of a horse, which died with symptoms of forage poisoning following the ingestion of moldy hay (?) gave the following results:

Brain, spleen and scrapings from the small intestines were negative for *B. botulinus*. Two samples of the intestinal wall, cæcum and colon, contained *B. botulinus*, type A, while one sample of the intestinal wall of the rectum, was negative. The soil on which the hay was grown contained the spores of *B. botulinus*, type A. Unfortunately no specimens of the moldy hay were available for feeding experiments.

(*e*) Oakdale: January–February, 1919, mouldy hay from a large stock supposed to have been the cause of forage poisoning in horses contained *B. botulinus*, type A. Reported by G. S. Burke, Jour. Bacteriology, 1919, IV, p. 551.

In several localities forage poisoning in horses antedated the occurrence of human and fowl botulism.

(5) *Colorado.*—An extensive outbreak, which affected 1,800 horses with a mortality of 1,000 during the months of July to September, 1919, was considered forage poisoning; sporadic cases have been numerous.

(6) *Connecticut.*—Numerous forage poisoning outbreaks have been reported, but "hemorrhagic septicemia" is suspected.

(7) *Delaware.*—Forage poisoning has been prevalent for many years; one county lost 100 horses in 1919.

(8) *Florida.*—Many sporadic cases.

(9) *Illinois.*—According to Graham in the Animal Pathology Exchange, July, 1921, the diagnosis of equine botulism was confirmed in two instances by a bacteriological examination; 36 outbreaks in cattle, sheep, swine, and horses have been recognized.

(10) *Indiana.*—No report available.

(11) *Kansas.*—Many single and group cases have been observed aside from the extensive outbreak in 1912. In three outbreaks an unsuccessful attempt was made to isolate *B. botulinus* from the feed, soil, etc. Healthy animals exposed to the same conditions as the diseased ones were immunized with *B. botulinus* antitoxin without effect.

(12) *Kentucky.*—No official reports available; numerous outbreaks are indicated from the publications of Graham.

(13) *Louisiana.*—In certain localities a disease resembling forage poisoning reoccurs annually; prophylactic *B. botulinus* antitoxic serum treatment is said to have produced satisfactory results. Consult also Bull. 106, Agric. Exp. Stat., Baton-Rouge, July, 1908.

(14) *Missouri.*—Reports an "immense" amount of forage poisoning several years ago.

(15) *Minnesota.*—Numerous outbreaks diagnosed as cerebrospinal meningitis, hemorrhagic septicemia, etc.; many cases have been observed, when animals are changed from dry feed to ensilage, particularly corn silage. Some cases developed in pastures subsequent to heavy rains.

(16) *Michigan.*—No official report, but Miss Lowe states in The Canner (1921, *52*, May, p. 31) that cases of forage poisoning from *B. botulinus* have occurred in the State. The toxicogenic bacterium has been recently demonstrated by the laboratory in silage.

(17) *Montana.*—Reports 17 outbreaks involving 1,004 head of cattle and horses and 3,000 sheep.

(18) *New Hampshire.*—Many sporadic cases and a few extensive outbreaks have been reported.

(19) *New Mexico.*—Several outbreaks have been observed.

(20) *New York.*—No report available.

(21) *New Jersey.*—An extensive outbreak occurred in 1912, near Cape May. Several sporadic cases have also been noted in pasture-fed horses. Graham and Schwarze (26) proved one spleen specimen of a horse to be contaminated with a *B. botulinus*, type A; they failed to isolate *B. botulinus* from a sample of hay obtained from one of the suspected pastures. The laboratory of the commission has made the following findings:

Cape May soil: *B. botulinus*, type B.

Soil on meadow, where horses died in 1912, and salt soil mixture: *B. botulinus*, type B.

Soil, Jones farm, southeastern part of New Jersey: *B. botulinus*, type B.

Soil, Westewelt farm, southeasterm part of New Jersey: *B. botulinus*, type B.

Soil, Johnson farm, southeastern part of New Jersey: *B. botulinus*, type B.

(22) *Nevada.*—Reports 29 outbreaks, several extensive, with reoccurrences on the same ranches. One outbreak was caused by "musty rotten" potatoes and one by garbage.

(23) *Oklahoma.*—Reports for 1920 an extensive outbreak in "river bottom lands;" sporadic cases are quite common.

(24) *Oregon.*—Numerous sporadic cases: one of five horses showed typical botulism at Klamath Falls, where several chicken outbreaks have occurred.

(25) *Pennsylvania.*—Reports isolated outbreaks and sporadic cases.

(26) *Texas.*—Forage poisoning prevalent in the Pecos Valley; moldy corn or cane is regarded as the causative food.

(27) *Washington.*—Reports many outbreaks. According to an investigation of the commission the following feeds were considered responsible:

	Instances.		Instances.
Moldy potatoes	15	Corn ensilage	1
Beet ensilage	4		
Moldy hay	2	Total	25
Rotten turnips	3		

(28) *Wyoming.*—Reports several sporadic outbreaks prior to 1919.

(29) *Virginia.*—Many outbreaks have been observed; moldy corn is considered as one of the factors in forage poisoning.

The data thus far secured and synthesized at least justify the conclusion, that forage poisoning, probably due to feeds other than preserved or fermented food, is not uncommon among horses of the United States. An approximate loss of 3,000 horses has been estimated for the five Western States, California, Colorado, Oregon, Nevada, and Montana, within the years 1915–1920. In certain localities the disease reoccurs annually and is of considerable economic importance. Moldy and spoiled hay, potatoes, corn, etc., are the feeds generally mentioned as the products carrying the disease producing poison. It is also stated that the rations may appear wholesome and yet be highly poisonous. However, nothing definite as to the nature of the toxic substance could be determined from the circular letter. Some of the observations in California are suggestive and support the laboratory examinations of Graham and his associates. *B. botulinus* has been isolated from the intestinal tract of two horses which died from forage poisoning in two separate outbreaks. On several occasions the same toxin type was demonstrated in the feed, but repeated attempts to prove the presence of *B. botulinus* toxin by feeding the saline extract of several pounds of suspected feed to guinea pigs were frankly negative or inconclusive. Graham had similar failures in administering to guinea pigs 1 to 2 cc. of water in which contaminated oats had been immersed. It is, however, mentioned that a horse consuming this water succumbed, showing the symptoms of botulism. These and similar observations suggest that horses may be more susceptible to *B. botulinus* toxin via the digestive tract than guinea pigs. Provided this statement is correct the presence of *B. botulinus* toxin in horse feeds must in the future be demonstrated by ingestion experiments on immunized and nonimmunized horses. It will probably be necessary to employ the same species of animal for subsequent experiments, to establish the influence of desiccation and light on the toxin of *B. botulinus* produced in damp hay or cereals. In fact, the entire toxicological problem must be studied on horses and it would appear economical if a number of States, which suffer from forage poisoning appoint a commission and pool their financial resources for a thorough unbiased study of this malady.

Among the various unknown causes which contribute to the generation of the botulism toxin in spoiled hay and vegetables, moisture and heat are probably the most essential, although sufficient time must elapse since the preceding harvest to permit the formation of the toxin. An inquiry into the seasonal distribution of forage poisoning in California revealed the fact that the disease increases during the fall and winter months. In Figure 10 an attempt has been made to parallel the monthly number of forage poisoning outbreaks with the monthly rainfall recorded for the last six years in the localities in which the disease occurred. During the months of October and November an average temperature of 71° F. prevailed in the southern section while a temperature of 55° F. was noted in the northern section of the State. The climatic conditions in form of early rains followed by warm weather in September and October were excellently suited for the growth of molds and bacteria and probably also *B. botulinus*. It is therefore of interest to note that nearly one-third of the forage poisoning cases were recorded for the months of September, October, and November. In other States the two factors arbitrarily chosen for consideration act at different seasons of the year, a fact which may explain the prevalence of forage poisoning either during the spring and early summer, or fall and early winter. Observations made in New Jersey, however, have shown that forage poisoning disappears after the first heavy frost. Inasmuch as the toxin of *B. botulinus* is resistant to cold and can be preserved in a frozen state for at least three weeks the above mentioned fact rather indicates that this poison is probably not the main cause of forage poisoning in New Jersey irrespective of the bacteriological demonstration of *B. botulinus* in the spleen of a horse by Graham and Schwarze and our own isolation of the bacillus from soil specimens. It is to be regretted that most of the reports received were sketchy in character and did not supply enough data to permit additional conclusions as to the etiological agent of food intoxications in horses.

Early in the course of the studies it was thought that the demonstration of *B. botulinus* in the intestines of horses or in the feed had some diagnostic significance and that it was indicative of equine botulism. Subsequent laboratory tests and in particular the isolation of *B. botulinus* from the tissues of cattle, which died from other diseases than botulism, seriously invalidated the original conclusions. Tests have been undertaken to establish in what proportion *B. botulinus* can be isolated from the viscera of slaughtered animals by the procedures customarily employed in the laboratory. Until these studies are completed or until other experiments have either demonstrated the presence of the preformed botulism toxin in the suspected feed or proven that the bacillus possesses invasive properties for horses, the numerous isolations of the organism from animal tissues or the apparently wholesome food can not be accepted as proof for the conclusion that certain forms of forage poisoning are actually equine botulism.

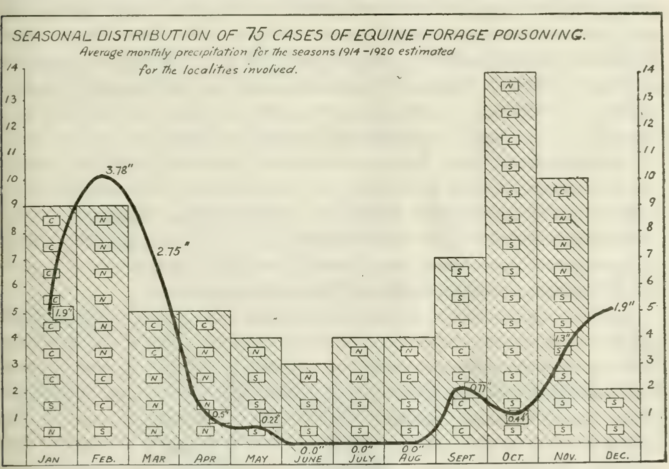

FIG. 10.

C. BOTULISM IN CATTLE.

Hart and Hayes (19) concluded from their experiments that cattle are resistant to *B. botulinus* toxin and probably could consume a very large amount by mouth without effect. Recently Graham and Schwarze (27) reported some observations on a sporadic toxemia in cattle which has some bearing on this subject. Several cattle of a herd consuming corn silage from which *B. botulinus* type B, was isolated, developed symptoms of forage poisoning on two different occasions and four animals died. The two workers concluded that *B. botulinus* toxin in the ensilage was primarily related to the disease in question. A feeding experiment on 43 cattle which had been injected with botulinus antitoxin was inconclusive, one animal, which did not receive antitoxin showed no visible illness. In this connection one recalls also the work of Theiler and his associates (28) on lamziekte, a disease of cattle in South Africa. Deficiency of phosphorous in the vegetation of certain areas during the drought season leads to the development of pica or craving for carrion, which contains a highly potent toxin. The "lamziekte toxin" resembles the toxin of *B. botulinus*, but it is stated that the symptoms of experimental botulism in cattle are distinctly different from lamziekte. Unfortunately the bacterium responsible for the "toxin of lamziekte" has not been isolated in pure culture.

In the light of these reports it is considered important to describe some observations collected by the commission. No attempt had been made to investigate systematically certain maladies of cattle which were suspected by veterinarians as botulism, but the findings to be reported were made accidentally and their bearing on the problem of bovine botulism could not be underestimated.

In 1914, one member of the commission (K. F. M.) (29), investigated in cooperation with the State veterinarian a fatal cattle disease which occurred in the mountainous regions of California and Nevada. The malady was characterized by a hemolytic jaundice, hemoglobinuria and extensive anemic infarcts in the liver. Attention was called at that time to the presence of various types of anaerobic bacteria in the liver lesions. Other work prevented further studies in California, but the late Dr. W. B. Mack and more recently Drs. E. Records and L. R. Vawter, of the University of Nevada, have diligently pursued research work in order to find the cause of the disease and to prevent the extensive losses which have been noted in Nevada. In January, 1921, Dr. L. R. Vawter discussed with members of the commission some of the anaerobes which he had isolated from the liver infarcts. Subsequently a culture of a toxic bacterium was sent to the laboratory of the commission and identified as *B. botulinus*, type A. On January 27, 1921, Doctors Records and Vawter with two members of the commission autopsied, near Reno, Nev., a two-year old heifer four hours after death. The animal showed the typical lesions of the ictero-hemoglobinuria disease. *B. botulinus*, type A, was isolated independently by two different laboratories from the liver lesions, lymphnodes, intestinal wall, and content. Fresh cattle manure and hay obtained from a haystack on the ranch gave cultures of *B. botulinus*. A third strain of *B. botulinus*, type B, was isolated from the tissues of a cow which had died from the same disease on a ranch many miles removed from the other two places where the previous fatalities had occurred.

These interesting observations prompted the commission to communicate with various stock owners of California who had lost cattle from the same disease during the last few years. Thus far no specimens have been submitted for examinations; but in February the laboratory of the Los Angeles Department of Health forwarded a broth culture containing the fragments of a liver infarct which had been removed from the cadaver of cow. This animal which was from a dairy farm near Los Angeles had shown all the symptoms of icterohemoglobinuria. *B. botulinus*, type A, was isolated from the specimen.

These findings of *B. botulinus* were considered very suggestive until Doctor Vawter informed the commission that he has not been able to again isolate the same organism from several cases of ictero-hemoglobinuria, which came subsequently under his observation. Moreover, Doctors Records and Vawter concluded from their experiments on cattle that in Nevada at least a certain type of *B. welchii* is responsible for the fatal cattle disease. A report dealing with their important observations on experimental botulism and the differences between this toxemia and ictero-hemoglobinuria has been recently published (Jour. Am. Vet. Assn., 1921, *60*, p. 155.) From the facts just presented it must be concluded that *B. botulinus* is not infrequently a common inhabitant of the intestinal tract of cattle; in the course or during the agonal stage of certain diseases it may even invade the tissues. It is only remotely possible, that these bacteria have an etiological relationship to the lesions from which they have been isolated. In fact it was because of the observations made on these cattle that the statement has been so frequently repeated in preceding chapters that the mere cultural isolation of *B. botulinus* can not be considered diagnostic of botulism.

D. BOTULISM IN HOGS.

Hogs display a marked resistance to poisoning from *B. botulinus* toxin experimentally fed in relatively large doses. In several reports it is mentioned that one or several hogs which consumed a considerable portion of poisonous home-canned food failed to show any symptoms of intoxication, although a number of chickens which ate some of the spoiled food developed fatal botulism. In the course of the investigations conducted by the commission a number of inclusive reports, which attribute several fatalities among hogs to the ingestion of a *B. botulinus* like poison in feeds, garbage, and spoiled home-canned products, have been collected. This information is tabulated in Table 14.

TABLE 14.—*Botulism in hogs.*

No.	Food responsible.	Number of hogs.	Location.	Date (year and month).	B. botulinus isolated.	Type.	Coincident cases.	Reported by—
1	Home-canned string beans	6 sick; no deaths	Yelm, Wash	1921, Feb	Jar of string beans	A	1 human, 20 chickens	Commission.
2	do	1 died	Decatur, Ind	1918, Feb	Jar of string beans and spleen of human case.	B	7 human	Decatur Democrat of Mar. 1, 1918.
3	Home-canned pears	15 died	Oroville, Calif	1917, Sept				Commission.
4	do	2 sick	Hollister, Calif	1918, Feb	Feces of hog 3 months after ingestion of suspected food.	B		E. C. Dickson, Arch. Int. Med., 1918, *22*, p. 494, and G. S. Burke, Journ. Bacteriol., 1919, *4*, p. 549.

REPORTED AS BOTULISM, BUT DIAGNOSIS NOT CONCLUSIVE.

No.	Food responsible.	Number of hogs.	Location.	Date (year and month).	B. botulinus isolated.	Type.	Coincident cases.	Reported by—
a	Beet ensilage	30 died	Yakima, Wash	1919, Nov				Commission.
b	Beet ensilage and moldy beets.	8 died of 25 sick	Artesia, Calif	1918, Dec				Do.
c	Commercial food	4 died	Chico, Calif	1920, Sept	B. botulinus demonstrated in feed.			Do.
d	Home-brew liquor	Several	Colusa, Calif	1919, Sept				Commission and F. Kelley in California State Board of Health Bul., 1919, *15*, p. 114.
e	Garbage	20 died	Perryville, Md	1920, June				Commission.
f	do	16 died	Reno, Nev	1920				Do.

In analyzing the data presented in Table 14 it will be noted that in only three instances was the diagnosis of botulism in hogs supported by bacteriological tests. In two outbreaks the animals recovered spontaneously, but *B. botulinus*, type B, was isolated from the manure of two hogs, which had ingested spoiled home-canned peas three months previous to the stool examination. In seven instances the owners or local veterinarians interpreted symptoms and lesions in a number of hogs to be those of porcine botulism and reported the outbreaks to the commission. Unfortunately there was no means of verifying the correctness of the diagnosis, and the facts are mentioned in order to stimulate further investigations. Until careful investigations have determined the relative susceptibility of swine to the toxin of *B. botulinus*, or until the presence of the poison in the suspected hog feed, hog tonic, etc., has been demonstrated experimentally, it must be concluded that botulism in hogs is very rare and that it has thus far only been proven following the ingestion of spoiled home-canned vegetables.

In this connection a recent publication of Graham (30) deserves some comment. In this paper it is reported that *B. botulinus*, type A, has been frequently isolated from certain local (intestinal?) lesions of pigs; these animals exhibited, following the injection of antihog-cholera serum and virus, symptoms of unthriftiness, muscular twitching, posterior paralysis, etc. Inasmuch as 16 per cent of the commercial hog-cholera serum and virus samples contained *B. botulinus*, it is concluded that the swine disease is probably hog cholera superimposed upon a specific intoxication due to *B. botulinus*. It is not stated whether the samples of hog-cholera serum and virus have been tested for the toxin of *B. botulinus* but is assumed that the bacterium possesses invasive properties and is capable of causing a specific low-grade intoxication in hogs. The data presented fail to warrant such far-reaching conclusions; in fact, they only confirm the well-known observation that *B. botulinus* is an inhabitant of the intestinal tract of hogs and that carelessly prepared hog-cholera serums may occasionally become contaminated with this organism.

E. BOTULISM IN DOGS, CATS, AND GOATS.

In report 22 it is stated that one dog which ingested a part of the discarded home-canned apricots responsible for five human cases and several chickens (report 57) exhibited for a few days the symptoms of posterior paresis, but ultimately recovered. The members of the commission have repeatedly observed similar symptoms in dogs experimentally fed or inoculated with type A toxin.

In connection with one chicken outbreak in Los Angeles (report 21, Table 12) it is authoratively stated that a milk goat which consumed about 1 quart of discarded home-canned string beans exhibited the symptoms of botulism. After an incubation time of two to three days the goat refused to eat; the milk secretion dropped to one-third of the usual amount collected; there was marked constipation and general weakness. Complete recovery took place toward the end of the second week, and four weeks later the animal gave birth to two healthy kids. The type of *B. botulinus* could not be determined. Four months after the consumption of the poisonous feed one examination of chicken feces and goat manure was negative for *B. botulinus.* It is not unlikely that the beans contained relatively little toxin. The housewife tasted some pods without consequences; six ducks, which ate a considerable portion of the spoiled food, remained well, and only six of ten chickens developed botulism. It is well known that goats are very susceptible to subcutaneous injections of *B. botulinus* toxin, and that great care must be exercised in the course of immunization.

Part VII.

FEATURES OF BOTULISM.

A. SYMPTOMATOLOGY AND COURSE.

When discussing the time of onset of symptoms of botulinus intoxication in human beings, it must be borne in mind that the victims of botulism fall into two main groups, viz, those in which there is initial acute gastrointestinal disturbance, followed by the typical botulism symptoms, and those in which the typical botulism symptoms are the first to manifest themselves. Approximately one-third of the recorded cases in this country in which data are available have shown symptoms of acute gastroenteritis type, nausea, vomiting, and diarrhea, which usually come on early, last for from 12 to 36 hours, and disappear with the onset of the typical symptoms of botulism; in the remaining two-thirds of the cases the evidences of nervous system involvement have been the first to appear. The acute gastroenteritis is apparently the result of local irritation by the spoiled food which is ingested, whereas it is now known that the typical botulism symptoms are the result of the action of the botulinus toxin upon certain portions of the peripheral nervous system (31).

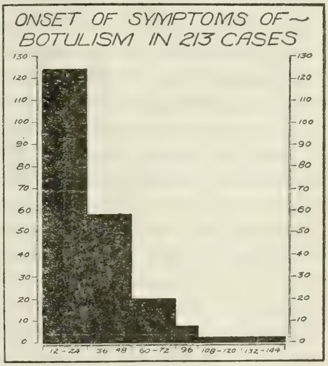

Fig. 11.

The onset of symptoms in botulinus intoxication is usually from 18 to 36 hours after the ingestion of the poison, although they may appear very much earlier or may be considerably delayed. The earliest onset recorded in the American literature is 2 hours (88) and, in this series, 4 hours after (66) the consumption of the spoiled food; and the most delayed onset in this series (54) occurred on the eighth day. In 74 per cent of the cases the onset of symptoms occurred within 48 hours (Figure 11), and in general it may be stated that when the symptoms occurred very early they were of the acute gastrointestinal type.

When gastrointestinal symptoms are present, the patient may complain of burning and distress in the region of the stomach, or there may be nausea and vomiting, with or without diarrhea, which may persist for a few hours or for a day or two. The characteristic effect of botulism is, however, an early inhibition of the gastrointestinal movements, which results in early and persistent constipation, and when initial diarrhoea is lacking the constipation may be one of the first symptoms noted. Frequently there is distention of the intestines, but there is rarely any pain, tenderness, or rigidity after the diarrhea has ceased. The earliest symptom in the majority of cases is a peculiar indefinite lassitude and fatigue, sometimes associated with dizziness or headache, which the patient often attributes to the accompanying constipation.

Disturbances of vision occur very early, and are frequently the first indication of serious illness. There may be initial scintillations and dimness of vision, due to early loss of ability to accommodate for near vision. There is early involvement of the third cranial nerve, and the occurrence of blepharoptosis, mydriasis, loss of reflex to light stimulation, and diplopia is practically constant. Occasionally the pupils may be irregular in contour and unequal. Complete loss of accommodation soon follows. Impaired function of the external rectus muscle appears earlier and more frequently than of the superior oblique muscle. Nystagmus and vertigo are not uncommon, and photophobia has been recorded in a few cases. Nystagmus may be seen on one side only.

The majority of authors agree that there is no disturbance of the retina. Apparently the dimness of vision is dependent upon loss of ability to accommodate, as usually the patient can distinctly see distant objects with either eye alone.

Coincidently with or closely following the onset of visual disturbances there is usually difficulty in swallowing and in talking, and frequently the patients complain of a sensation of constriction of the throat. The tongue appears to be larger than usual, is sluggish in its movements, and heavily coated on the surface although the edges may be clean. There may be complete paralysis of the pharyngeal muscles with loss of the pharyngeal reflex. The voice is low and without normal tone and eventually there is complete aphonia. Even in the milder cases the onset of early fatigue of these muscles is very evident, and after short attempts at conversation speech becomes noticeably slower and more difficult and the voice more husky.

The difficulty in swallowing is apparently largely due to impaired function of the pharyngeal muscles, as the patients state that they have difficulty in forcing the food from the pharynx into the esophagus but that if it is once started they can easily swallow. It is probable, however, that the dryness of the mouth and throat is a contributing factor, as in the milder cases the patients are able to swallow solid food if they take a drink of liquid. Attempts to swallow frequently induce strangling spells which may be very severe, and may persist until the patient is exhausted. Regurgitation of fluids through the nose and insufflation into the trachea and bronchi frequently occur during the strangling spells.

General muscular weakness, especially weakness of the muscles of the neck, is most striking. In severe cases the patients lie with the muscles completely relaxed and are unable to raise their arms or legs from the bed, but although such a condition closely simulates paralysis it is seldom that true paralysis of the skeletal muscles is observed. There is often incoordination of the muscular movements of the arms and legs, and the patients may have difficulty in picking up small objects and may walk with an atoxic gait but the reflexes of the skeletal muscles are practically always intact.

A very striking characteristic of botulinus intoxication is the almost complete absence of sensory disturbances. There may be initial headache and gastric distress but usually the patient does not suffer any pain. Occasionally there is tinnitus and partial deafness, and a few cases have been reported in which there was numbness and tingling of the extremities, but in by far the greater number of reported cases it is noted that there was no disturbance of sensation.

Mentality usually remains clear until within a very short time before death. Restlessness, insomnia and occasionally hysterical attacks are noted, particularly at the onset, but the patient often becomes apathetic or even somnolent as the intoxication increases. There may be spells of irritability when he is aroused, apparently induced by his inability to make himself understood, or to swallow, and often there is great apprehension of approaching death. Occasionally there may be coma for a short time before death, and a few cases are recorded in which there were convulsions, but a most striking feature of the intoxication is that mentality is usually unimpaired throughout the illness.

Inhibition of many of the secretions is also characteristic and the patients complain bitterly of dryness of mouth, pharynx, and nasal passages. A few cases have been reported in which the salivary flow was increased, and it is said that this is usually true in forage poisoning in horses, but in the cases examined during this investigation the serous portion of the saliva has always been inhibited and there has always been a thick tenacious mucus in the pharynx which is removed with the greatest difficulty.

According to the literature there is usually inhibition of sweat secretion, but it is not uncommon to find the patient bathed in a profuse sweat, which has an offensive odor. When sweating is inhibited the skin becomes hard and dry.

There is considerable variation in the reports concerning the secretion of urine, but apparently in the majority of cases the amount of urine secreted depends directly upon the amount of fluid which the patient has received. Oliguria and anuria have been described, but that is not surprising when one considers the difficulty or impossibility of swallowing liquids. There has been no characteristic change in the urinary constituents or sediment in the cases of this series.

The temperature is usually subnormal. In the majority of cases it ranges between 96° and 98.6° F., and in uncomplicated cases remains low during the whole illness. Not infrequently there may be fever during the last days of the intoxication, but this is usually a sign of the development of broncho-pneumonia.

The pulse rate may be slow in the early stages of the intoxication, but later in the course of the illness becomes rapid. It is not uncommon to observe a rate of from 100 to 120 per minute and in some cases of as much as 150 or 160 per minute. The action of the heart muscle is impaired; there may be no visible cardiac impulse and the heart sounds are feeble and distant. The combination of subnormal temperature and rapid pulse is most striking.

There is often no disturbance of respiration in the early stages of the intoxication, but towards the end breathing becomes difficult and labored, and there may be extreme dyspnoea and irregular breathing, sometimes of the Cheyne-Stokes type, with all the accessory muscles brought into play. Later, as fatigue of the respiratory muscles develops, there may be partial or complete asphyxia and the most frequent immediate cause of death is respiratory failure.

There are very incomplete data concerning the results of the ordinary clinical laboratory tests in botulinus intoxication. As was stated above, examination of the urine shows no characteristic changes from normal. Blood counts have been made in a few cases without revealing any typical blood picture, although the number of cases in which examination has been made is much too small to justify any definite conclusions.

In this series there are records of 8 cases where red blood counts were made and in 7 of them the count was between 5,200,000 and 5,600,000 per c. c. In 15 cases leucocyte counts are recorded and the count varies from 7,000 to 20,400 with 4 below 10,000, 7 between 10,000 and 14,000, and 4, 16,000 or more. In all but two where differential count was recorded the polymorphonuclear neutrophiles were between 70 and 85 per cent. In at least one case where the leucocyte count was over 16,000 necropsy revealed the presence of broncho-pneumonia.

Examination of the cerebrospinal fluid has been recorded in four cases. In one instance it was stated that there were 80 cells per c. c., but the type of cells was not specified. In the remainder the fluid was reported normal.

Blood-pressure records in six cases showed systolic pressures between 120 and 140 mm. and diastolic pressures of from 80 to 90 mm. In another case it was recorded that the blood pressure was normal and in two that the systolic pressure was 120.

The duration of illness in fatal cases varies greatly, although the majority of victims die in from three to six days after ingesting the poisonous food. In a series of 173 fatal cases where data were available, 18 patients died within 48 hours after the food was eaten, and 1 survived for 26 days; but 117 persons, or 67.6 per cent, died in from three to six days after ingesting the poison.

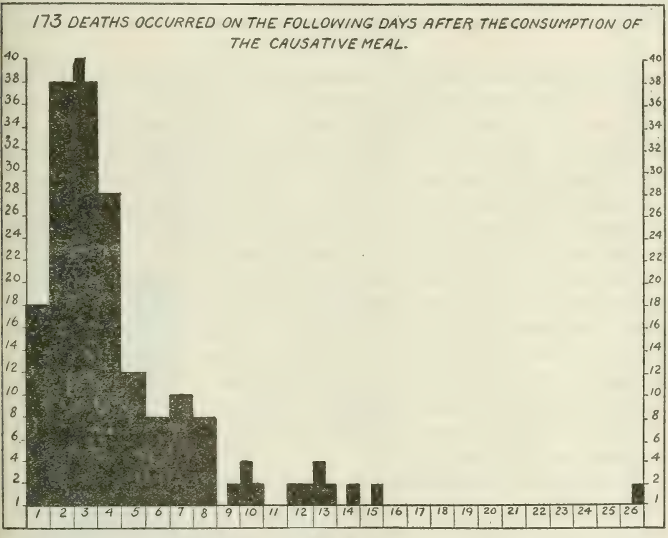

FIG. 12.

Death usually occurs from cardiac or respiratory failure. It has been frequently observed that the heart continued to beat for several hours after voluntary respiration had ceased, and cases are recorded when artificial respiration has maintained life for several hours after voluntary respiration had ceased. Usually there is terminal asphyxia with cyanosis, and occasionally the patient dies in a strangling spell. It is not uncommon that there may be apparent improvement in the general condition of the patient but that death results from insufflation broncho-pneumonia.

When recovery occurs convalescence is extremely slow and tedious. The severity of the illness usually reaches its maximum within 10 days of the poisoning and improvement follows slowly.

The strangling and the difficulty in speaking and swallowing frequently improve first, but the muscular weakness and the disturbances of vision disappear more slowly. Many cases are recorded in which the patients were comparatively well within two or three months, but the weakness, vertigo, and emaciation may persist much longer. The disturbances of vision in some cases are the last to clear up and may persist for months. There appears to be no permanent severe disability, although some patients complain of constipation after recovery has taken place.

B. MORTALITY.

The recorded mortality of botulism in the United States is very high, 61.7 per cent, and is surprisingly uniform in the various areas included under Sections III, IV, and V, viz, California 61.3 per cent, Washington 69.1 per cent, and other portions of the United States and Canada 60.9 per cent. There is very great variation in the mortality of different group outbreaks, however, ranging from zero in report 21, 10.3 and 12.5 per cent in reports 63 and 10, respectively, to 100 per cent in reports 59, 60, and 62, etc.

In a series of 246 cases where data were available and of which 173 resulted fatally, it was found that 147, or 85 per cent, of the fatal cases were persons in whom the onset of symptoms occurred within 48 hours after ingestion of the poison. In many outbreaks there is indication that the time of onset of symptoms is directly dependent upon the amount of poison ingested, and this observation shows that the severity of the illness and the mortality rate is also directly dependent upon the same factor.

A comparison of the mortality among patients who have an initial acute gastrointestinal disturbance before the onset of the typical botulism symptoms, with those in whom the botulism symptoms are the first to be manifested, indicates that, contrary to expectations, the initial vomiting and purging does not materially affect the outcome of the intoxication. All the records of cases in this country do not contain the necessary data, but in 180 cases where data were available it was found that 71.8 per cent of those who suffered acute gastrointestinal disturbances and 73.2 per cent of those in whom there was no initial nausea or vomiting resulted fatally.

C. PATHOLOGY.

There is no characteristic gross lesion by which botulism can be recognized at necropsy. The only constant finding is the marked congestion of the central nervous system and of the abdominal and of thoracic viscera. There may be multiple hemorrhages around the base of the brain and upper part of the cord, and the brain tissues may be edematous and show scattered hemorrhages. The lungs are congested and often show areas of broncho pneumonia. The heart muscle is soft and flabby, the liver, kidney and spleen are hyperemic and all the parenchymatous organs are opaque and cloudy on section. There may be ecchymoses and hemorrhages on the serous surfaces and in the lungs.

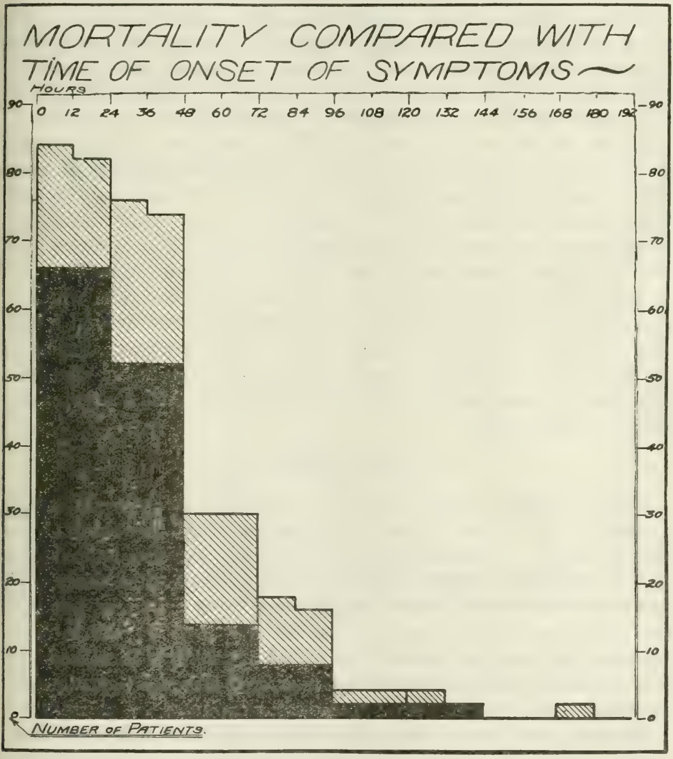

FIG 13.

On microscopic examination, all the tissues show marked congestion, and often the arteries, veins, and capillaries are all engorged with blood. In the brain and meninges there are numerous larger or smaller perivascular hemorrhages around the distended vessels, and in the brain and other organs, especially in the lungs, there may be large hemorrhages which extend beyond the immediate vicinity of the vessel.

The occurrence of cellular thrombi of the type first described by Ophüls (32) are very often found in the tissues of patients in whom the progress of the intoxication has not been too rapid, but they may not occur when the duration of the illness has been very short. When present, they are very suggestive that death has been caused by botulinus intoxication, but, as was recently pointed out by one of us, (Dickson, Journal, A. M. A., 1921, LXXVII, 483), they can not be considered pathognomonic of botulism. In a series of 12 necropsies of human victims of botulism, the tissues of 9 contained the typical thrombi, and of a large series of experimental animals which survived longer than 48 hours after the administration of toxin the tissue of the great majority also contained the thrombi.

In none of this series of human victims of botulism, nor of animals in which the poisoning was experimentally produced, has there been any indication of destruction of the ganglion cells such as was first described by Marinesco (33). Neither has there been any indication of perivascular infiltration with lymphocytes such as is characteristic of epidemic encephalitis, save in one case which has been recorded (Geiger, Public Health Reports, July, 1921) and in which a very few lymphocytes were seen in the areolar tissues around a few of the blood vessels in the brain. There should be no confusion in the microscopic diagnosis between botulism and epidemic encephalitis, since perivascular lymphocytic infiltration is not a characteristic lesion of the former.

D. SERUM THERAPY.

Nothing definite can be said as to the value of antitoxin in the treatment of botulism, although thus far no satisfactory results have been obtained. It has been shown by experiment that animals can be fully protected if sufficient antitoxin is given simultaneously with or within a short time after the administration of the toxin, but that the proportion of the fatalities rapidly increases as the interval between the administration of toxin and antitoxin is increased. A few cases were noted where animals recovered although symptoms of poisoning had appeared before the administration of the antitoxin.

Complete data concerning the administration of botulinus antitoxin to human victims of botulism in the United States are not available, but there are at least eleven outbreaks in which it has been used. In two instances it is known that a type B antitoxin was used in what was later proved to be a type A intoxication, and in three others the details concerning the administration of the antitoxin have not been published. There are six outbreaks, however, in which homologous antitoxin was administered, and concerning which the details of its use are known, and these afford the only reliable data at hand upon which to base any conclusions as to its efficacy.

In these outbreaks (reports 3, 5, 7, 15, 68, and 69) 31 persons were ill and 23 died. Seventeen of the patients died before antitoxin was available and 6 succumbed after receiving the injections. One person recovered without having been given antitoxin and 6 recovered after it had been administered.

The mortality in these outbreaks was 74.2 per cent.

In only three of the outbreaks (reports 3, 5, and 69) did any of the victims recover after receiving the antitoxin, and analyses of these show that in every instance the more severely poisoned persons had died before the antitoxin was administered, and that those who were the more seriously ill when they received the antitoxin succumbed in spite of its use. In no instance was antitoxin available until at least four days after the poisonous food had been eaten, and in one instance it was not administered until the ninth day.

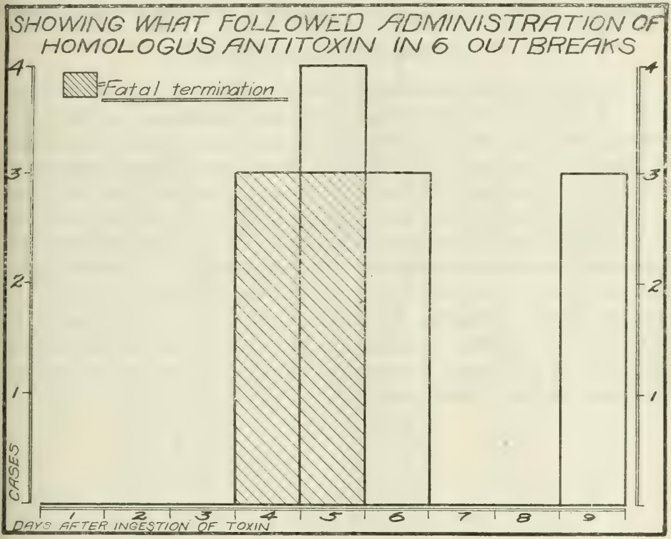

FIG. 14.

The only conclusion that can be drawn from these observations is that botulinus antitoxin is of no value as a therapeutic agent when given late in the intoxication. There has been no record of its administration sufficiently early to enable any comparison with results which can be obtained at will under experimental conditions, and none to indicate that it would not prove efficacious if given early in the intoxication.

In all instances of botulinus intoxication, therefore, the antitoxin should be administered as soon as possible after the consumption of the poisonous food. Time may be gained by using polyvalent serum or a mixture of both types of serum until such time as the type of the toxin has been determined. It should be given intravenously, after the patient has been tested for hypersensitiveness, and, if necessary, desensitized. Large doses, at least 20,000 units, should be given, and since it seems probable that there is no continuous formation of toxin after the poisonous food has been ingested, a single administration of the whole amount of antitoxin is preferable to repeated small doses.

E. BACTERIOLOGICAL DIAGNOSIS.

From the foregoing discussion it is evident that a provisional diagnosis of botulism is often made without difficulties. In fact, the commission has in 61 instances been obliged to recognize the disease by the clinical records of the cases or by the coincident occurrence of botulism in chickens. However, from a medicolegal and also from an epidemiological standpoint, the accuracy of these diagnoses can in some instances be seriously questioned. An attempt should therefore always be made to determine the true nature of the poisoning by bacteriological examinations. Furthermore, in connection with the specific serum treatment, information relative to the type of *B. botulinus* intoxication necessitates the application of laboratory methods. According to a summary presented in Table 15, the following procedures have been employed:

TABLE 15.

No.	Report.	Food and location.	Date.	Toxin in food.	Isolation of B. botulinus.	Type of isolated strain.	Laboratory work conducted by
1	71	Blood sausage, Massachusetts	1912	Not tested	From sausage		E. M. Randall.
2	73	Cottage cheese, New York	1914	Emulsion 48 hours, incubated at 37°, killed guinea pig within 36 hours.	From cheese	[1] B	Laboratories New York State Department of Health.
3	66	Home-canned corn, Oregon	1915		Gizzard of 1 chicken	A	E. C. Dickson.
4	1	Home-canned beans, California	1916		Crops and gizzards of 3 chickens	A	Do.
5	70	Ham, Florida	1916	Scrapings of ham from around bone produced symptoms in cat and death of 1 dog.	Ham emulsion		E. M. Randall.
6	2	Home-canned string beans, California.	1917		Jar of beans found in cellar, same lot that had caused botulism in 7 persons.		E. C. Dickson.
7	6	Home-canned corn, California	1917		Jar of corn		Bureau of communicable diseases, California State Board of Health.
8	47	Home-canned asparagus, Washington.	1917		Crops and gizzards of 7 chickens	A	E. C. Dickson.
9	15	Home-canned apricots, California	1918		Gizzard of 1 chicken; cultures of spleen and contents of intestine of human case negative.	A	Do.
10	69	Home-canned string beans, Indiana	1918	Liquor surrounding beans nontoxic	Beans and mangoes; spleen of 1 case	B	R. Graham, University of Illinois.
11	67	Home-canned asparagus, Idaho	1919	Not tested	Salad, juice and cap of 1 jar	A	Bureau of Chemistry, U. S. Department of Agriculture.
12	57	Commercially canned olives, Ohio	1919	M. L. D. olive brine=0.001 cc., 0.15 cc by mouth.	Olives and brine; also 5 other jars of 46, same lot.	A	Ohio State University and Bureau of Chemistry.
13	58	Commercially canned olives, Michigan.	1919	0.01 cc., M. L. D	Olives and brine	A	H. W. Emerson, Hygienic Laboratory, University of Michigan, and Bureau of Chemistry.
14	61	Stuffed olives, Montana	1919		Dried pimento stuffing adherent to empty bottle.	A	Bureau of Chemistry.
15	72	Canned sausage, Wisconsin	1919		Sausage meat		F. E. Church, Milwaukee.
16	59	Commercially canned olives, New York.	1920	0.004 cc. by mouth killed mouse in 72 hours.	Olives and brine	A	New York City Board of Health, Department of preventive medicine, Harvard Medical School.
17	7	Minced olives, California	1920	M. L. D. 0.1 cc. of macerated relish	Macerated relish, cultures of contents of colon, ileum, spleen, liver, and lungs of 1 human case negative.	A	Bureau of Chemistry, western division, and commission.
18	60	Commercially canned olives, Tennessee.	1920	0.01 cc. brine caused death of guinea pig in 9 hours.	Olives and brine	A	B. W. Fontaine, Memphis Medical College.
19	68	Commercially canned beets, Arizona.	1920	Washings of dry can nontoxic	Jejunal wall of 1 case on 7th day after eating beets.	B	Commission.

[1] (Nevin strain).

No.	Report.	Food and location.	Date.	Toxin in food.	Isolation of B. botulinus.	Type of isolated strain.	Laboratory work conducted by
20	3	Commercially canned spinach, California.	1920	Food not available	Stool of 1 patient on 6th and 7th days; of another on 12th day after eating spinach.	A	Commission.
21	63	Commercially canned spinach, Michigan.	1921	Fatal to guinea pigs and chickens	Liquor and spinach leaves of numerous cans of same lot; stool of 1 case on 11th day of illness and another on 31st day; negative.	A	R. Graham, Michigan Food and Drug Department, and commission.
22	64	do	1921	do	Liquor and spinach leaves of numerous cans of same lot.	A	Do.
23	65	Commercially canned spinach, Indiana.	1921	do	do	......	R. Graham, University of Illinois.
24	46	Home canned string beans, Washington.	1921	0.01 cc. of 1:10 saline extract of 1 bean fatal to guinea pig in 28 hours.	From 1 bean	A	Commission and Bureau of Chemistry.
25	50	Home canned corn, Washington	1921	......	From remainder of another jar of same pack.	A	Commission.
26	62	Commercially canned olives, Pennsylvania.	1921	0.001 cc. brine	Brine and olives	A	Commission and Bureau of Chemistry.
27	4	Commercially canned spinach, California.	1921	Container dry; enrichment gave positive culture.	Washings of can and stool of case on 6th day of disease.	A	Commission.
28	18	Home canned fish	1921	Not available	Crops, gizzards, and intestines of 2 chickens.	A	Do.

(*a*) *Determination of the presence of B. botulinus toxin in the remnants of the causative food.*—In 11 outbreaks (reports 7, 46, 57, 58, 59, 60, 62, 63, 64, 65, 70) the presence of the toxin of *B. botulinus* has been proven by subcutaneous or intraperitoneal inoculations or by feeding of guinea pigs, mice, chickens, cats, and one dog. These tests have been very thorough in the more recent outbreaks caused by ripe olives and spinach; for example, the M. L. D. for guinea pigs of the olive brine has been definitely determined. Such tests should in the future be conducted without delay in order to procure the urgently needed information relative to the average toxicity of spontaneously spoiled vegetables and fruits responsible for human botulism. In several histories it is indicated that a definite relationship exists between the onset and severity of the symptoms, the mortality and the amount of poisonous food consumed.

Aside from establishing the nature of a human intoxication it is of extreme importance that information relative to the type of *B. botulinus* intoxication be made available at the earliest possible moment. The benefits of a therapeutic application of a specific antitoxin depend on an early administration and instead of wasting precious time it is recommended that either a polyvalent serum or an equal amount of both types of antitoxins be inoculated as soon as a proper diagnosis has been made.

The antitoxin prepared against each of the types A and B is specific for the homologous toxin and will not protect against the heterologous one. These type determinations of the poisonous food have thus far been conducted on guinea pigs and mice. Quite recently Graham and Schwarze (34), suggested the feeding of the suspected or responsible food to chickens as a preliminary method of determining the type of *B. botulinus*. They found that type A is fatal when fed to mature chickens, while type B does not produce illness in chickens even when fed in liberal amounts. The commission has had no opportunity to verify this work by feeding spoiled food, which was derived from human or animal outbreaks, but it was pointed out in the chapter on chicken botulism that spontaneous type B intoxication of chickens has been observed.

For a rapid method of determining the types of toxin the methods described by Bengtson (35) and Orr (36) can be recommended if any of the spoiled food is still available. By the inoculation of three series of three mice, each one series with culture, vegetable liquor, fruit juice, etc. (1, 0.5, and 0.1 cc.), alone, one previously inoculated with type A antitoxin and another with type B antitoxin, it may be possible to determine within a short time which type is the causative organism and therefore which type of antitoxin should be used for treatment. Mice are more favorable for carrying out these tests, for the reason that symptoms usually develop more rapidly and more characteristically.

(*b*) *Isolation of B. botulinus from the suspected food.*—In six outbreaks (reports 6, 67, 71, 72, 73) the food which caused the poisoning was either not tested for the toxin of *B. botulinus*, or the liquor surrounding the vegetables was innocuous to guinea pigs (report 69), or the can contained only dry fragments of the suspected material (reports 4, 61, and 68) and in two of these cases (reports 4 and 61) *B. botulinus* was isolated in shake or in enrichment cultures. The types were determined either from the filtered enrichment or from the purified cultures. Methods excellently suited to this purpose have been described in a recent publication (37).

In two outbreaks (reports 2 and 50) the bacteriological diagnosis was made from jars of home-canned vegetables of the same lot that had caused the poisoning.

In seven single of group outbreaks (reports 1, 3, 15, 18, 47, 66, and 68) the remnants of the suspected food were not available and the laboratory resorted to three indirect methods, namely the isolation of *B. botulinus* from the cadavers of poisoned chickens, or the stools of human cases or the intestinal tract or spleen of fatal cases. The first named procedure has already been discussed in the preceding chapter and it was emphasized that in the light of recently acquired knowledge these indirect findings are of limited value. The other two methods deserve, however, some consideration.

(*c*) *The isolation of B. botulinus from the stools of clinical cases.*—Stool specimens of four clinical cases (reports 3, 4, and 63) obtained from three different outbreaks contained *B. botulinus*, type A, on the sixth, seventh, eleventh, and twelfth days, respectively, after the consumption of the causative meal. One specimen each procured from a case on the thirty-first day (report 63) and another from a case on the twenty-sixth day of illness (report 81) were negative. The diagnostic value of the demonstration of *B. botulinus* spores in the fecal remnants of the causative meal, which consisted of spinach in the few positive cases, has been strengthened by additional observations of the commission. Fifty stool specimens of people who had ingested raw vegetables and fruits purchased in the open market proved negative for *B. botulinus*, although repeated examination of the fruits and vegetables these people ate had shown the presence of the spores of *B. botulinus*. Additional tests with several hundred samples from children from rural districts are still in progress of investigation. Inasmuch as constipation is almost a constant manifestation of botulism, it appears quite possible that positive stool cultures may be obtained from convalescent patients for at least two or perhaps three weeks.

On several occasions agglutination tests with the serum of convalescents have been carried out. Various strains belonging serologically to different groups are clumped in dilutions as high as 1:100. Numerous normal human serums gave identical reactions. Thus it would seem that serum agglutination tests can not be used for diagnostic purposes. (See Armstrong, Story, and Scott, Public Health Reports, 1919, *34*, p. 2893.)

Miss Bengtson (35) has suggested the intraperitoneal inoculation of white mice with the citrated blood of persons suffering from botulism as a possibility of establishing a differential diagnosis or to determine the type of intoxication. Kob (38) in 1905 reported that the serum from a patient collected on the ninth day after eating poisonous food caused typical symptoms when injected into white mice, and recently Semerau and Noack (39) in Strassburg demonstrated by the inoculation of guinea pigs the presence of toxin in the serum of four patients on the fourth, sixth, ninth, sixteenth, and even on the twenty-fifth day after the consumption of the poisonous food. In America this procedure has only been tried on one case (report 80) with a negative outcome. These tests are of considerable clinical and scientific importance and should by all means be repeated when the occasion arises.

(*d*) *The isolation of B. botulinus at necropsies.*—Systematic necropsy cultures by means of dependable enrichment mediums have not been carried out. The commission has studied the tissues of four cases (reports 7, 15, 41, and 68) and has isolated *B. botulinus* from the jejunal wall of one case fatal on the fifth day of the disease (report 68) and the brain tissue of another (report 41). The demonstration of a type-B strain in the spleen of one case (report 69) has been reported by Graham. Culturing of tissues like brain, etc., with negative results are mentioned in reports 80 and 81. In this connection it is noted that the paresis of the intestinal tract favors the escape of intestinal organisms into the blood and lymph stream. The report of the bacteriologist and pathologist who made the examination of the brains of two cases involved in outbreaks 63 and 64 describes the presence of *B. welchii* in these tissues. The finding of *B. botulinus* in the spleen of fatal cases must probably also be attributed to either an ante or a post mortem invasion. Members of the commission were not successful in three attempts (reports 7, 15, and 68) to isolate *B. botulinus* from the spleen. In all instances the organs had been removed soon after death.

The demonstration of *B. botulinus* in the stools of clinical cases suggests an examination of the colon and the rectum. In cases which end fatally on the second to fourth days, enrichment cultures, with large samples of the duodenal, jejunal, and ileac chyme or contents, should be carried out. From the standpoint of early diagnosis or in medicolegal cases it is possible that the presence of the ingested toxin might be demonstrated in vomitus or contents of the stomach by the inoculation of laboratory animals with filtrates.

(*e*) *The types of B. botulinus responsible for botulism in the United States.*—In Table 15 the bacteriological findings of the 89 reports are briefly summarized. *B. botulinus* has been isolated from the food or the stools of clinical cases or at necropsy or from the organs of chickens in 28 (31.4 per cent) instances. The toxins or the isolated strains have been typed in 23 outbreaks and 19 cultures have been identified as type A, while only 3 have been proven to be type B. To this list can be added 10 type A and 4 type B isolations from chickens, 6 type A and 4 type B from the feed and the tissues of cases of equine "forage poisoning;" 3 type A and 1 type B, from organs of cattle and one type B from the manure of a recovered hog. Graham (40) states in one of his publications that type B is more frequently encountered in animals than type A. Unfortunately his published records do not contain the necessary data and it has therefore been possible to include in this comparison only the reports of six definitely identified isolations. The relative proportions of the two types isolated from various materials connected with human or animal botulism in the United States is briefly as follows:

B. botulinus, type A	38
B. botulinus, type B	13
Total	51

The significance of these findings is not entirely clear. Both types of *B. botulinus* occur in the soil of the United States; but it has been noted that certain localities particularly the Middle Western and Eastern States furnish more frequently soil cultures which contain a type B toxin. The mutual relationship of the A to the B types is still unknown, but some incomplete observations indicate that *B. botulinus* found in virgin, unfertilized soil is usually type A. However both types have occasionally been isolated from one and the same specimen. The entire problem considering the importance of the various types of *B. botulinus* will be discussed in a paper dealing with the distribution of the spores of this organism in nature.

PART VIII.

GENERAL DISCUSSION.

In the present chapter the data at the disposal of the commission are used for a consideration of three phases of the botulism problem, which are of general interest to the clinician, the packer of food and the epidemiologist. The subjects to be discussed will be treated under the following headings: (1) *B. botulinus* infection versus intoxications due to preformed toxins; (2) The economic importance of spoilage due to *B. botulinus* in home and commercially canned food; and (3) A comparison of the food products responsible for botulism in the United States and Germany.

A. B. BOTULINUS INFECTION VERSUS INTOXICATION DUE TO PREFORMED TOXINS.

The original conception of van Ermengem, who considered *B. botulinus* a toxicogenic saprophyte has recently been questioned by Orr (41) and by Edmondson, Giltner, and Thom (42). These workers found that guinea pigs receiving large numbers of toxin free spores by way of the digestive tract, usually died in from two to four days. Control pigs injected with antitoxin lived. These experiments deserve confirmation; in fact it is by no means proven that large numbers of spores are detoxified by a simple heating to 80° C. for 15 minutes. Laboratory tests have verified the facts established by the above workers, in that guinea pigs developed symptoms of botulism and died after the ingestion of enormous quantities of washed spores, but it was found that in these instances the spores had not been completely detoxified. Further experiments showed that after heavy spore emulsions have been treated with specific antitoxins repeatedly washed and heated at 80° C. in order to destroy the adherent traces of antitoxin they are innocuous to guinea pigs when administered via the digestive tract. Until further experimental studies on other animals are completed it is advisable to scrutinize the various botulism outbreaks for evidence, which would suggest that the spores *B. botulinus* may germinate and produce their toxin in the human body. V. Burke, Elder, and Pischel (43) have carefully analyzed the available information and have come to the conclusion that a true botulism infection following the ingestion of toxin free spores never occurs in human beings. This view is supported by several observations of the commission.

The spores of *B. botulinus* are widely distributed in nature. In certain localities numerous people probably ingest daily spores of *B. botulinus* on raw vegetables and fruits. If the intake of a small number of spores causes an infection, numerous cases of botulism without a history of preserved or canned food having been eaten a short time before the onset of the symptoms, should be on record.

The epidemiologic data of the numerous carefully investigated cases of botulism indicates that the intoxication has always been preceded by the ingestion of toxin-containing preserved food. In four instances, moreover, it is proven that the eating of uncooked spoiled food caused botulism while the ingestion of portions of the same food after cooking provoked no symptoms. These reports deserve detailed description.

Report 17.—A woman tasted a few beans of a jar of home-canned string beans, which exhibited no evident signs of spoilage. She developed fatal botulism. The entire contents of the jar was consumed after boiling for 35 to 50 minutes by three sons without any ill effects.

Report 26.—A mother died of botulism after drinking the fluid from a freshly opened can of home-perserved asparagus. The son escaped illness although he ate a portion of the asparagus after it had been cooked.

Report 47. A portion of a jar of home-canned asparagus was cooked on Sunday. None of the persons who ate the cooked asparagus suffered any ill effects. On Monday evening the remainder of the asparagus was "warmed up" by placing the jar for a few minutes in warm, not boiling water. The vegetables did not taste good but were eaten by one person, who developed botulism and died on the fifth day.

Report 53. A housewife tasted sour-tasting home-canned corn; she developed botulism, but recovered. Some of the spoiled upper part of a jar was thrown out and caused botulism among the barnyard fowls. The remainder of the jar was boiled for 30 to 40 minutes and was consumed by two childrem without any ill effects.

These histories indicate that boiling will as a rule destroy the toxin in the spoiled food, and prevent the development of human botulism. It may also be assumed that the persons, who ate this cooked and spoiled food ingested large numbers of spores without evil consequences. This deduction is, however, not entirely proven. It is known that in a suitable medium comparatively few *B. botulinus* organisms may generate a very potent toxin; in fact the number may be so small that even the usual excellent methods of plating or shake cultures fail to demonstrate the presence of *B. botulinus* (13). Furthermore, it has been repeatedly noted that the heating of spores of *B. botulinus* in fruits or vegetable juices at a temperature of from 80° to 100° C. causes a marked numerical reduction in viable spores. It is therefore quite possible, that the cooking of the spoiled food not only destroyed the poison, but also reduced the viable spores below the number, which as claimed by Orr, and by Edmondson, Giltner, and Thom, is capable of causing a true infection. The experiences gained from the study of past botulism outbreaks, therefore proves that thorough boiling of spoiled food prevents botulism but they do not support or refute the experimental results of the investigators mentioned.

However, in this connection, it should be mentioned that only prolonged boiling until all parts of the food have been maintained at the boiling point for the required length of time (30 to 40 minutes), will detoxify the spoiled food. Numerous epidemiological observations throw considerable doubt on the repeatedly published statements that suspicious canned goods when emptied into a kettle and boiled for five minutes can be eaten without danger. Randall (44) in 1912, had already made the observation that the lethal properties of *B. botulinus* toxin are not impaired by a temperature of 212° F. maintained for 10 minutes. Burke, Elder, and Pischel found spoiled string-bean juice may still be toxic after boiling for 7 minutes, and in order to be safe the spoiled food must be boiled for at least 15 minutes. Laboratory tests conducted by the commission have confirmed these facts, and have shown that the toxin in the vegetable tissues and juices is more resistant to heat than toxin in broth or meat cultures. This difference is probably not entirely explained by the variable heat penetration of vegetables and broth cultures. It is recalled that spoiled string-bean liquor, M. L. D. 0.0001, may remain toxic after 15 minutes boiling in an open kettle.[7] Furthermore, the liquor of a spoiled 2½ can of spinach vigorously boiled for 20 minutes proved fatal to guinea pigs on subcutaneous inoculation, and when fed to the same species of animal caused typical botulism in three days. Depending on the bulk of the spoiled food, size of the kettle and numerous other uncontrollable factors, complete detoxification can usually only be accomplished after boiling for at least 30 to 45 minutes, depending on the altitude. Furthermore, the escape of gas may simulate boiling and may be quite vigorous at temperatures below the boiling point. On account of these uncertainties one is forced to demand with unremitting insistence that food preserved by heat whether home or commercially canned must be processed in such a manner as to ensure either absolute sterility or at least complete destruction of *B. botulinus* in the product, and the thorough boilings of any carrying the slightest suspicion of spoilage.

B. THE ECONOMIC IMPORTANCE OF SPOILAGE DUE TO B. BOTULINUS IN HOME AND COMMERCIALLY CANNED FOOD.

From the numerous sensational statements issued in the daily press, the medical journals and the popular magazines one could conclude that spoilage due to *B. botulinus* is a common every day condition of preserved vegetables and fruits. In fact the impression is being disseminated that certain food products, particularly ripe olives and spinach, are especially liable to be contaminated with the poison, no differentiation being made between home and factory canned food products. Botulism can be prevented, provided the public is fully acquainted with the facts. In this connection it is advisable not only to point out the sources and the character of the danger, but also to restore confidence in the use of canned foods. The data at the disposal of the commission are excellently suited for the purpose, and this chapter is not written to incriminate or to exculpate any particular product, but simply for the purpose of segregating and placing on record all the facts.

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[7] Recent studies of material obtained from outbreak 90 have shown that the liquor (M. L. D. 0.0001) obtained from a jar of home-canned asparagus was *not detoxified* after being heated in a deKhotinsky bath for *30 minutes at 80° C.* in sealed and open tubes.

It has been previously noted that in 51 instances spoiled home-canned vegetables, fruits, or animal products have been responsible for human botulism in the United States. That the methods which are usually employed in canning vegetables and fruits at home are not always efficient is proven by the frequency with which a larger or smaller proportion of the finished products becomes spoiled. An approximate estimate of spoilage due to *B. botulinus* can be made from the various outbreaks of botulism among barnyard fowls, which developed symptoms of botulism or died following the consumption of poisonous spoiled food. According to Figure 15 there are on record a total of 87 instances in which home-canned spoiled string beans (40), corn (21), asparagus (7), etc., contained *B. botulinus* and its toxin. It is probably impossible to determine accurately the annual amount of home canning done in the United States, and the above figures are therefore only of relative significance.[8] A certain percentage of the home-canned products are discarded by the housewives every year, and it is impossible to estimate how many jars or cans contained the fatal poison. From the facts which can be definitely established at this time, it seems, however, not unlikely that the number of home-preserved products which contained the poison is relatively small. This conclusion is based on certain data collected in California. According to a report of the State home demonstration leader, 1,934 women members in the farm bureaus of 11 counties canned during 1920 the following amount of fruits, vegetables, and meats:[9]

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[8] It has been estimated by Doctor Farrell of the U. S. Department of Agriculture that in 1917 more than 850,000,000 glass jars of food had been put up at home in addition to all which had been put away in tins. In this connection it is recalled that the yearly commercial pack of fruits and vegetables, exclusive of fish, meats, milk, etc., is approximated at more than 1,099,000,000 cans or containers: in fact, it is known that annually more than 1,000,000,000 cases of commercially preserved food products are placed on the market.

[9] For comparison it is considered advisable to record as far as possible some figures which will give an idea of the extent of the commercial canning carried out in California. According to the report of the Canners League of California for 1920, the packers preserved the following amounts of vegetables and fruits:

Vegetables:	Cases.	Fruits:	Cases.
Asparagus	1,024,813	Free peaches	1,547,687
Peas	86,074	Cling peaches	5,205,511
Tomatoes	1,858,822	Pears	1,184,288
Tomato products	833,019	Apricots	2,312,020
String beans and other vegetables	881,385	Cherries	647,977

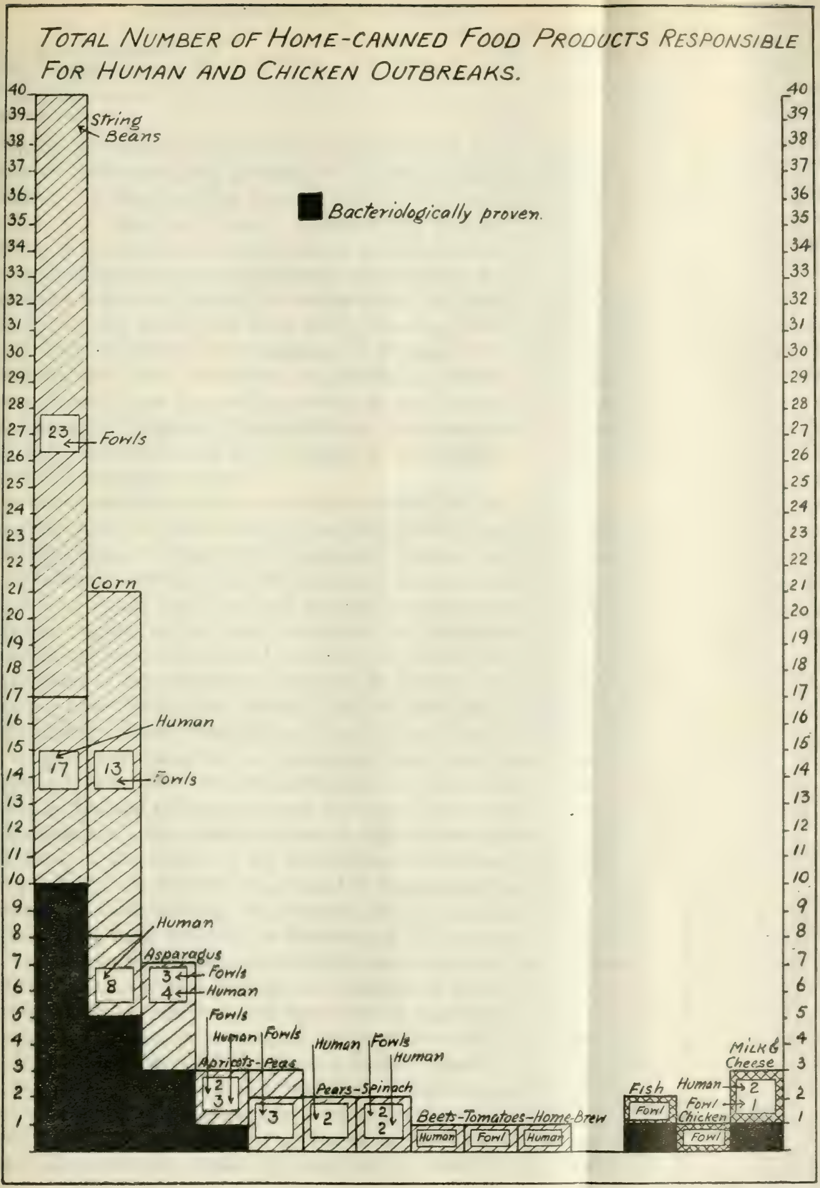
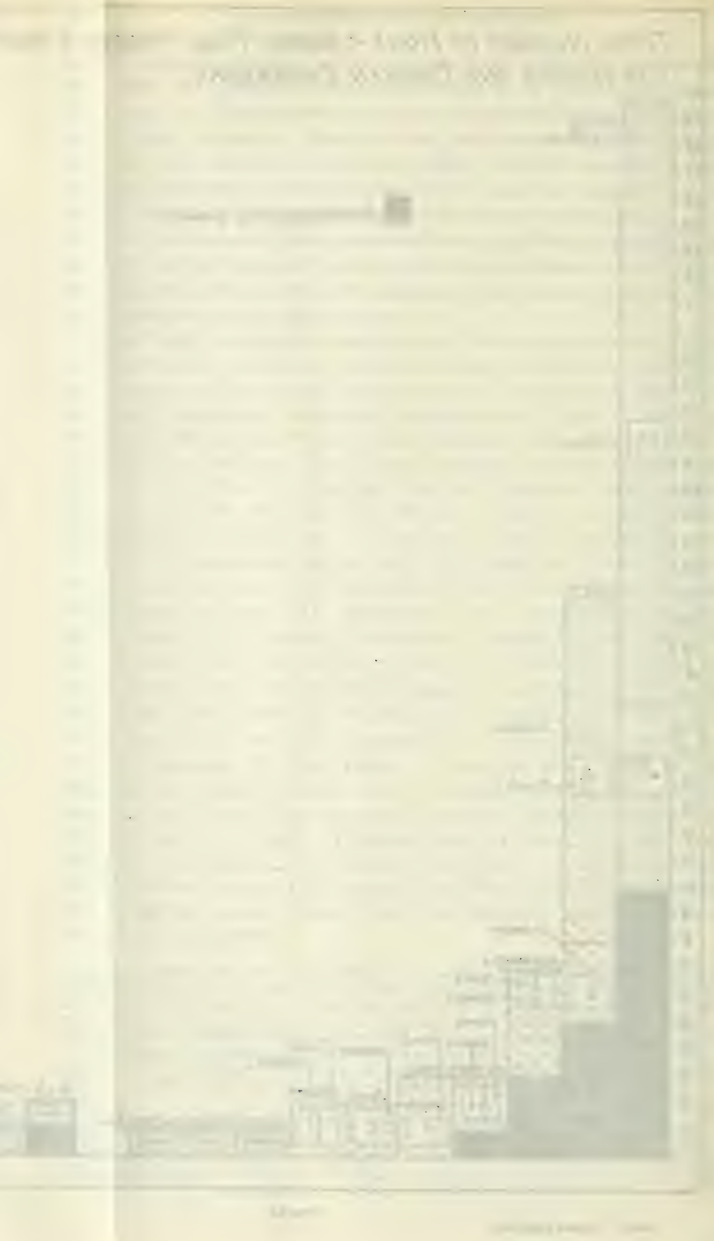


FIG. 15.

4794. (To face page 102,)



Quarts of fruits preserved	19,946
Quarts of vegetables	9,804
Pounds of meat canned	1,136
Pounds of fish	170

During the last 10 years not more than 100 jars have been proven either on epidemiological grounds or by bacteriological methods to contain *B. botulinus* and its toxin. In some of the instances only one jar out of as many as twenty contained the poison. Even if one doubles or quadruples the above figure and for the sake of discussion assumes arbitrarily that per annum an average of 200 quarts of vegetables were toxic, it will be evident that less than 0.02 per cent of the total pack underwent some form of spoilage which was dangerous for human beings. This estimate is probably too high, but the figures indicate that even where *B. botulinus* is widely disseminated in nature and quite frequently present on raw fruits and vegetables and where the problem of botulism has been essentially associated with the consumption of home-canned food, spoilage of vegetables due to this organism is rare.

From the available facts it can be concluded that this form of food poisoning may be prevented by an educational campaign which should emphasize that successful canning of foods in the home depends on cleanliness, soundness of raw material, and thorough processing. There is no doubt that the usual processing temperatures employed in home canning of ordinary vegetables are inadequate. In fact, spores of the recently isolated strains of *B. botulinus* will resist the temperature of boiling water for several hours and, as far as recorded cases go, these spores pass through the ordinary processes of household canning without destruction. In order to avoid spoilage due to *B. botulinus* it is important to recommend only those procedures and methods which take into consideration the heat penetration and acidity of the food to be canned and the spore resistance of the toxicogenic organism. The determination of standardized processes, which will, even in the hands of an inexperienced housewife, preserve a food product from *B. botulinus*, should be practicable, and there is no occasion for discontinuing the preservation of food at home. The method to be used can only be determined by systematic studies of the various physical factors involved in home canning, and by using scientific principles and methods in working out these processes. A revision of the various methods now in use is urgently needed, but it is suggested that new procedures be recommended only after the biology of the spores of *B. botulinus* has been carefully investigated.

Until 1916, only three or four instances of botulism poisoning have been attributed to commercially canned goods, as against a much larger number traced to food prepared in the household, but the tabulated records in Figure 16 show that *B. botulinus* has exerted its activity in the products of the canning factory. In 31 instances commercially packed or preserved foods are suspected of having contained the toxin of *B. botulinus*. In 14 outbreaks the poisonous character of the factory-canned products was toxicologically and bacteriologically proven, while in 13 instances these foods are under grave suspicion, and in 3 outbreaks the available evidence is inconclusive.

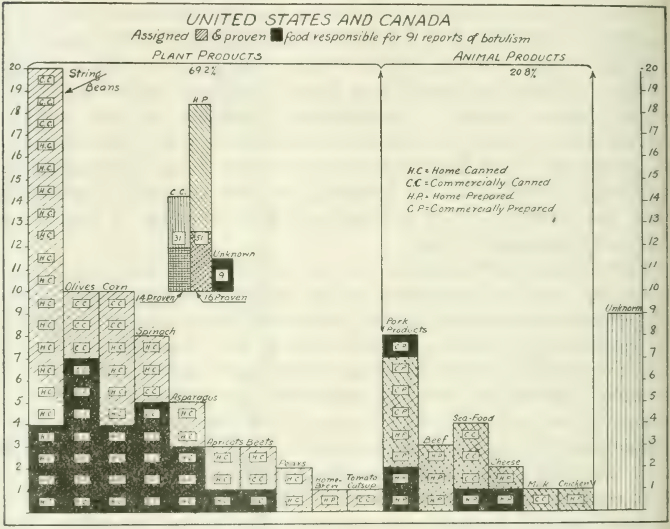


Fig. 16.

Among the commercially canned products which caused 22 of the outbreaks, ripe olives (10) and spinach (6) are mainly mentioned. Certain questions naturally suggest themselves, namely: (1) Is botulism spoilage a frequent or a rare occurrence in factory-canned pickled olives and spinach? (2) Is there any possibility that this form of spoilage may increase in the future? (3) What are the causes and how can *B. botulinus* be eliminated from commercially canned goods?

For more than a generation California has distributed millions of containers of ripe olives and there has never been any indication until 1919 that this food in a preserved state may contain the poison of *B. botulinus*. In the course of the investigation conducted by the Bureau of Chemistry and by other agencies into the causes of this spoilage, it was found that less than a dozen, possibly 9 or 10 containers of ripe olives and 3 cans of minced olives, contained the fatal toxin. In Table 16 the figures dealing with the annual production of ripe olives in California are compared with those of toxin containing jars or containers. It is obvious from this comparison that the actual number of toxic cans is indeed very small and the facts available never supported the sensational statements which had been made in the daily press. The number of cases of botulism poisoning caused by ripe olives and the mortality is no higher than has occurred in many outbreaks from home-canned products. Inadequate sterilization, combined with the practice of holding olives during the war years in water or weak brine solutions, were undoubtedly the causes responsible for *B. botulinus* spoilage. The various agencies which investigated the olive industry agree that efficient sterilization of fresh and carefully handled raw products will prevent further outbreaks of botulism.

TABLE 16.

		Poisonous jars.
1916.	Olive pack consisted of 6,472,000 cans and containers	0
1917.	Olive pack consisted of 5,184,000 as recorded cases go	1
1918.	Olive pack consisted of 6,480,000 as recorded cases go	8
1919.	Olive pack consisted of 7,848,000 as recorded cases go	5
1920.	Olive pack consisted of 3,288,000 processed according to the regulations of the California State Board of Health.	

The six outbreaks due to commercially canned spinach have been traced to four or five different factories. Three outbreaks were caused by cans of spinach which came from two inadequately processed packs of vegetables. It is now known that nearly 70 per cent of the cases of one spinach pack had to be discarded on account of spoilage.[10] Over 45 per cent of the cans examined contained viable bacteria and the liquor of 27 per cent of the swelled cans were toxic for guinea pigs. The actual number of toxin containing containers of spinach is not known, nor is it possible to determine the number of cans which are involved in report 64; hence a comparison similar to that given above for ripe olives would not be supported by facts.

In this connection it must be stated that the yearly average production of canned spinach exceeds 530,000 cases or more than 12,000,000 cans, and if one excludes the two obviously spoiled lots mentioned above one can only conclude that the toxin-containing cans of spinach represent an insignificant part of the total yearly pack. This, however, does not mean that every precaution against its occurrence should not be taken. The studies of various laboratories have thrown some light on the causes responsible for the survival of *B. botulinus* in canned spinach. Underprocessing of poorly blanched leaves in heavily filled containers (97 ounces "put in") must be mentioned as the main factor. Until botulism cases were attributed to canned spinach the packer was mainly concerned in reducing the percentage of spoilage and did not realize that *B. botulinus* must be considered.

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[10] See p. 42.

This he attempted to accomplish but failed to establish by carefully planned experiments, the process temperatures and the length of time necessary to secure actual sterility of the product. The available evidence indicates that the danger of botulism poisoning due to spinach can be eliminated by processing No. 10 cans, with a fill of 82 ounces and an initial temperature of at least 110° F, at the center of the can, at 252° F. for at least 90 minutes.

In reviewing the various factors which are regarded as conducive to *B. botulinus* spoilage in factory-canned pickled olives and spinach, incomplete sterilization must be considered the most important. Shortly before extensive outbreaks of botulism poisoning due to commercially canned products were reported, Weinzirl (45), and later Cheyney (46) showed that certain classes of canned goods on the market are frequently not sterile and that the proportion of cans containing viable aerobic spores varied according to the product, but might in some products reach as high as 25 per cent. Cheyney says in this connection:

> The more difficult the packers have found it to process a product the more contamination we have found in the cans of that product. This logically leads to the inference that some canned foods at present are being underprocessed and that the usual methods of processing must be increased to obtain actual sterility in these foods.

In the light of these findings it is not surprising that occasionally *B. botulinus* toxin should be found in commercially canned goods. Raw food material contaminated with the spores of *B. botulinus* may be subjected to the ordinary factory processing without the destruction of the spores. The surviving spores may remain latent in the canned food on account of the injury received during processing; they may be unable to germinate on account of the inhibitive action of the fruit and vegetable juices or they may grow and produce a swelling of the cans which causes them to be discarded as not marketable. On the other hand, it is known that canned goods may be nontoxic and yet when carefully cultured show viable spores of *B. botulinus*. These observations may be explained by the fact that certain chemical factors in form of concentration, reaction, and composition of the preserved food exert an inhibitive influence on the spores. In a few cases on record it is proved that they grew and produced a potent toxin with so little visible spoilage that it was unrecognized and was responsible for the poisoning of human beings.

It is unquestionably true that in certain areas agricultural products are more heavily contaminated with the spores of *B. botulinus* than in others, and that botulinus spoilage can therefore not entirely be prevented by careful selection of clean and sound raw material. From a practical standpoint, therefore, it is unfortunately impossible to depend on the action of the inhibitive factors to prevent the formation of toxin when viable spores remain within the container. As a rule the methods are sufficient to ensure freedom from the spores of *B. botulinus*, but the packer should aim wherever possible at absolute sterility of his food products. In this connection it should be noted that the usual understerilization which is met with certain vegetables like corn is mainly due to very resistant thermophiles, which do not grow at ordinary temperatures. From the data presented in previous chapters it is quite evident that the commercial methods have been generally sufficient and that with the exception of pickled olives and spinach understerilization for the spores of *B. botulinus* has been very rare. The danger from this source has now been fully recognized and scientific canning methods are replacing empiricisms. The methods to be chosen for the destruction of the spores of *B. botulinus* are being rapidly developed and it is confidently expected that *B. botulinus* spoilage in commercially canned food will be entirely eliminated in the very near future. The regulations of the California State Board of Health dealing with the packing of pickled olives and the agreement among the California packers of spinach are clearly indicative of the progress which is being made in this direction.

C. A COMPARISON OF THE FOOD PRODUCTS RESPONSIBLE FOR BOTULISM IN THE UNITED STATES AND IN GERMANY.

The recent publication of L. Bitter (47) dealing with botulism in Germany offers an opportunity to compare the causes of the disease as found in the United States with those established for Germany.

It will be noted from Figures 16 and 17 that in America canned vegetables and fruits were primarily involved, while in Germany animal products, particularly pork and fish, carried the fatal poison. Food products which were either preserved by inadequate pickling or by incomplete impregnation with the antiseptic substances of wood smoke or by short boiling and frying caused the majority of botulism poisoning cases in Germany. The only German outbreak which was definitely traced to canned string beans is for various reasons of considerable interest and therefore reviewed in detail.

The Alice Cooking School in Darmstadt preserved some string beans and served a portion in form of a salad at a regular dinner. Fifty-two persons partook of the food; 22 showed severe symptoms of botulism and 11 died. One man who consumed the cooked beans was not ill. The beans exhibited little physical disintegration, but had an abnormal odor. Landmann (48) demonstrated in the remnants of the string beans the toxin and isolated *B. botulinus* in culture. Gaffky (49), who received two empty and one unopened cans of string beans of the same lot, found also an aerobic, butyric acid producing bacterium. The isolated organism resembled *B. botulinus* and produced very potent toxins. The Konserven Zeitung, or trade journal of the packers, expressed in 1904 the opinion that the fatalities which occurred in Darmstadt were caused by bacterial toxins of *B. proteus mirabilis* and *vulgaris*, not those of *B. botulinus*, and it is claimed that several workers were able to support this contention by laboratory tests. In 1910 it was demonstrated by Leuchs (50) that the strains of *B. botulinus* isolated by Gaffky differed immunologically from van Ermengem's original culture. On a comparative basis it appears not unlikely that Gaffky's culture was identical with what is known to-day as a *B. botulinus*, type A, and if so, is probably the only type A culture which has been isolated in Europe. In Germany, botulism is usually caused by food which has not been subjected to severe processing temperatures and, with the exception of the Darmstadt outbreak, the studied cases, as far as they are published, were caused by a homologous group of strains: van Ermengem (51) noted that the spores of his culture were killed by heating to 80° C. for one hour. On the other hand, botulism in America follows the use of canned foods, which as a rule contain type A toxin. The spores of the type A strains resist the temperature of boiling water for a considerable period. Recent studies have shown that the soil of continental countries contains mainly type B strains, while the soil of the American States which had most of

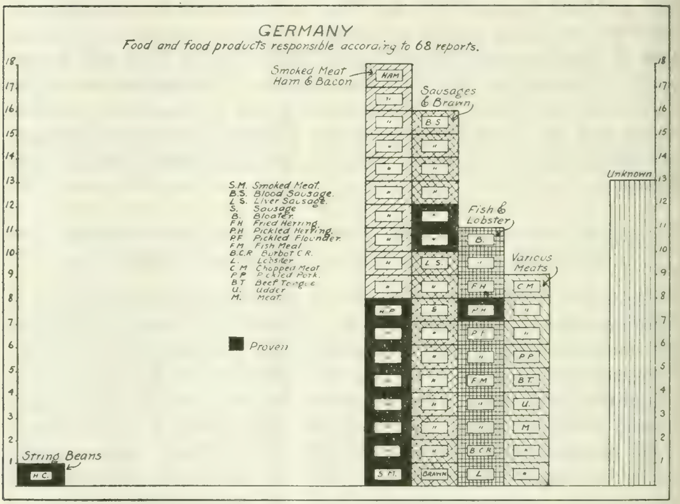

FIG. 17.

the human and animal botulism harbors primarily type A. It therefore seems possible that the absence of botulism in canned food in Europe is either due to higher processing temperatures or to lower heat resistance of the prevailing spores of *B. botulinus*. This suggestion deserves laboratory investigation.

The food and food products responsible for botulism in Germany were primarily home preserved. It is also pointed out that the prevalence of botulism in country districts frequently is due to inadequate preservation on account of the careless and unsanitary treatment to which the raw material is subjected by the rural population. Home slaughtering and preservation of pork products in form of sausages is so universally practiced in Germany that it is not at all surprising to find that about one-half of the botulism outbreaks were caused by this type of food. Inasmuch as many of the German records and histories are rather vague and indefinite, it seems, however, hardly fair to draw further comparative deductions.

PART IX.

CONCLUSIONS.[11]

(1) Outbreaks of botulism in the United States, following the consumption of toxin containing food products are not infrequent, but as a cause of death this intoxication is by no means as important as the numerous publications in the medical journals would imply. A total of 91 reports dealing with single or group outbreaks of botulism have been collected by the commission. In 30 instances only has the clinical or the post-mortem diagnosis been confirmed by toxicological tests. The following food products have been proven or assigned as the causes for the intoxications:

Home-preserved products.—51 outbreaks; 16 proven.

Home-canned string beans: 17 instances; 4 proven. (Food spoiled and bad odor, 7; bad taste, 4; no signs of spoilage, 3; unknown, 3 outbreaks.)

Home-canned corn: 9 instances; 4 proven. (Spoiled and bad odor, 6; taste, 2; not known, 1 outbreak.)

Home-canned asparagus: 5 instances; 3 proven. (Spoiled and bad odor, 3; taste, 1; no signs of spoilage, 1 outbreak.)

Home-canned apricots: 3 instances; 1 proven. (Spoiled, 1; bad taste, 2.)

Home-canned pears: 2 instances; none proven. (Spoiled, 1; bad taste, 2.)

Home-canned spinach: 2 instances; none proven. (Spoiled, 1; no sign of spoilage, 1.)

Home-canned beets: 1 instance; none proven. (No sign of spoilage.)

Home-brew liquor made from spoiled home-canned fruits and vegetables: 1 instance; not proven.

Cottage cheese: 2 instances; 1 proven. (Putrefactive odor, 1; spoilage unknown, 1.)

Home-pickled mackerel and herring: 1 indirectly proven. (Spoiled.)

Home-cured ham: 2 instances; 1 proven. (Not spoiled, 1; unknown, 1.)

Blood sausage: 1 instance; proven. (Spoilage unknown.)

Salted pork: 1 instance; not proven. (Spoilage unknown.)

Beef products: 3 instances; inconclusive, not proven. (Spoiled, 1; not spoiled, 1; spoilage unknown, 1.)

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[11] The deduction drawn from reports 90 and 91 are included.

Minced chicken: 1 instance; inconclusive.

Commercially preserved and canned products.—31 outbreaks; 14 proven.

Commercially canned underprocessed spinach: 6 instances; 5 proven. (Visibly spoiled and odor, 2; no signs of spoilage, 4.)

Commercially packed minced olive relish: 3 instances; 1 proven, 2 indirect. (Bad odor, 1; no signs of spoilage, 2.)

Commercially pickled and bottled ripe olives: 7 instances; 6 proven, 1 not proven. (Visible spoilage, 4; bad taste, 1; no signs of spoilage, 1; unknown, 1.)

Commercially canned beets: 2 instances; none proven, 1 indirectly. (Bad taste, 1; no signs of spoilage, 1.)

Commercially canned string beans: 3 instances; none proven, 1 inconclusive. (Spoiled, 1; unknown, 2.)

Commercially canned corn: 1 instance; not proven. (Bad taste.)

Commercially packed tomato catsup: 1 instance; not proven. (Unknown.)

Commercially canned pork and beans: 1 instance; inconclusive. (Spoiled.)

Commercially prepared sausages: 2 instances; inconclusive, not proven. (No sign of spoilage.)

Commercially prepared ham: 1 instance; not proven. (Bad taste.)

Commercially bottled clams: 2 instances; not proven. (Unknown.)

Commercially canned tuna fish: 1 instance; not proven. (Spoiled.)

Evaporated milk: 1 instance; not proven. (Spoiled.)

In recent years a striking increase of botulism has taken place in various localities, and foodstuffs, which were considered safe from *B. botulinus* deterioration have been found to be the cause of this type of poisoning. Laboratory studies have also demonstrated that *B. botulinus* is more widely disseminated in nature than has been generally suspected. Botulism can not be considered primarily a problem of California, the Pacific Coast States or the western slope of the Sierra Nevada and Rocky Mountains. The organized work in California enabled the commission to investigate sources of information, which were not accessible in other States. It is not unlikely that some of the essential findings determined for the Pacific coast could be duplicated by the activities of a similar commission in other localities.

(2) Since 1889 a total of 45 instances of human botulism have been reported for the State of California. A total of 132 people with 81 deaths are involved in these observations.

(3) There is no evidence of any special incidence at any age period. The sex distribution for 115 cases can be summarized in the following formula:

Children: Males: Females: = 1:3.13:3.46.

(4) The intoxication rate is very high; as a rule all who ate the poisonous food became ill. The available data do not permit of a comparison of the infectivity rate of food poisoning with the intoxication rate of botulism. The number of fatal cases varies greatly in different outbreaks. The case mortality rate for 129 cases in California is 61.3 per cent. For the children the mortality rate is higher than in adults.

(5) More than one-half of all the botulism outbreaks of California have occurred during the fall and winter months, namely October to February. In contrast with the bacterial food infections, which are usually prevalent during the hotter months botulism bears no relation to the climate, because it is as a rule intimately connected with the consumption of preserved food, which takes place during the winter season.

(6) Twenty-eight or 62.3 per cent of the outbreaks in California were caused by plant, while 11 or 24.4 per cent were attributed to animal food products; in 6 or 13.3 per cent of the outbreaks the carrier of the toxin was unknown; 13 outbreaks or single cases were caused by home-canned string beans, 2 by commercially packed spinach, 1 by commercially pickled ripe olives, and 3 by commercially prepared minced olive relish, 3 by home-canned apricots, 2 by home-canned pears, 1 by home-canned asparagus, 1 by home-brew liquor, 1 by home-canned and 1 by commercially canned corn, 3 by home-preserved beef products, 1 by commercially preserved and 1 by home-preserved pork products, 2 by commercially bottled clams, 1 by commercially packed pork and beans, 1 by commercially packed tuna fish, 1 by home-pickled mackerel and herring, and 1 by cottage cheese. In 12 instances commercially packed and in 27 observations home-canned products were involved.

(7) The prevalent idea that the food must always be visibly spoiled in order to be toxic is not entirely supported by the available observations, although there is no doubt that five single or group outbreaks were caused by home-canned string beans, which were spoiled by appearance, odor, or taste. The home-canned apricots and pears responsible for outbreaks were either moldy or visibly spoiled and had a sharp bitter taste. In two instances the home-canned corn and asparagus were very distinctly spoiled and emanated an offesive odor. In one report dealing with home-canned corn it is mentioned that the product was not spoiled but tasted "metallic." *It is definitely stated that two jars of home-canned string beans responsible for two outbreaks were not spoiled either by odor or taste.*

Commercially packed spinach was spoiled in one and appeared normal in taste and odor in another outbreak. The commercially canned minced olives connected with two single or group outbreaks were bitter in taste and on standing emanated a definite odor. No signs of spoilage were noted in one can of the same product, although it contained toxin. In the majority of instances the canned food is so spoiled as to attract the attention of the one who opens the container, but it should be emphasized that in four instances (home-canned string beans, commercially packed spinach and minced olives) macroscopic spoilage in form of abnormal consistency or appearance was not visible. *B. botulinus* spoilage can therefore not always be determined by the appearance or odor of the food.

(8) The frequent occurrence of botulism in the southern section of California is closely connected with the distribution of *B. botulinus* spores in nature, particularly in the soil.

(9) An investigation in the State of Washington revealed 13 outbreaks of human botulism (inclusive reports Nos. 90 and 91). A total of 26 people with 18 deaths are involved in these observations. A careful survey in the Yakima Valley demonstrated that there have occurred in this locality during the last five years, 8 outbreaks of human botulism, 27 outbreaks in domestic fowls, 26 in horses, 3 among cattle, and 1 among hogs. The mortality rate among the human cases was 69.1 per cent. Aside from being an important public-health problem, botulism assumes in the Yakima Valley also an economic aspect. Home-canned corn, home-canned asparagus, and home-canned spinach were the foods responsible for botulism. A single case possibly due to commercially canned evaporated milk has been investigated.

(10) Since 1912 a total of 33 instances of human botulism have been reported for the entire United States and Canada, exclusive of the States of California and Washington. A total of 187 people with 114 deaths are involved in these observations. The case mortality rate is 60.9 per cent. Twenty-four or 72.4 per cent of the outbreaks were caused by plant, while 7 or 27.3 per cent were attributed to animal products. In 16 instances commercially preserved foods were involved, namely, pickled ripe olives, 6 (all proven); spinach, 4 (3 proven); string beans, 2 (not proven); beets, 2 (1 proven indirectly); tomato catsup, 1; and sausage, 1 (inconclusive). The following home-preserved foods are mentioned in the reports: String beans, 4 (1 proven); corn, 3 (1 proven); asparagus, 1 (proven); beets, 1 (not proven); cured ham, 3 (1 proven); blood sausage, 1 (proven); cottage cheese, 1 (proven); and minced chicken, 1 (not proven). The commercially preserved foods originated in the following States: California, 9 (pickled ripe olives and spinach); Kansas, 1 (string beans); Ohio, 1 (beets); Wisconsin, 1 (sausages). In the majority of the reports it is stated that the canned food was noticeably spoiled either in appearance or at least in odor. Canned spinach and home or commercially preserved beets may show very little spoilage, although they may contain a very potent toxin. The causative foods were

either improperly preserved or underprocessed (ripe olives and two packs of commercially canned spinach, home-canned string beans, corn, and asparagus).

(11) Botulism is not uncommon among barnyard fowls in urban and rural poultry flocks. One hundred and six observations indicate that spoiled home-canned plant products as string beans, corn, asparagus, peas, spinach, apricots, and fish are primarily responsible for the intoxications. The diagnosis has been supported by bacteriological examinations in 25 instances. In 3 cadavers of chickens obtained from four different outbreaks the presence of *B. botulinus* toxin was demonstrated by the inoculation of guinea pigs with the filtered saline extract of the crop content. *B. botulinus*, type B, was found in four outbreaks either in the causative food or the crop of the chickens.

(12) Certain forms of "forage poisoning" in horses are probably true equine botulism. Preserved spoiled vegetables or ensilage caused four different outbreaks; the presence of *B. botulinus* and its toxin was proven indirectly by immunization experiments or by the coincident occurrence of botulism in human beings or fowls. Until experimental studies have demonstrated the presence of the preformed *B. botulinus* toxin in the suspected feed the numerous isolations of this organism from the cadavers of horses or the suspected wholesome or spoiled feed possess no diagnostic significance for equine botulism. This statement applies in particular to those cases of forage poisoning in which as causative feed, moldy hay, corn, potatoes, etc., are suspected.

(13) *B. botulinus* is in certain localities an inhabitat of the intestinal tract of cattle. In the course or during the agonal stage of some diseases it may invade the tissues. These bacteria have probably no etiological relationship to the lesions or to the diseases from which they have been isolated. The occurrence of spontaneous botulism in cattle has not been proven.

(14) Botulism in hogs, dogs, cats, and goats is very rare and has been noted following the ingestion of poisonous home-canned string beans, peas, and possibly pears.

(15) A typical case of human botulism is usually characterized by delayed onset, with or without initial acute gastro-intestinal disturbance, marked general muscular weakness, disturbances of vision with diplopia and blepharoptosis, dryness of the mouth and pharynx, accumulations of thick mucus in the pharynx, loss of ability to swallow and talk, strangling spells and regurgitation of fluids through nose on attempts to swallow, and persistent constipation. There is rarely any pain or disturbance of sensation. Mentality remains clear. The pulse rate steadily increases as the intoxication progresses, and the temperature is usually subnormal.

Death usually results from respiratory failure but insufflation pneumonia is not an uncommon cause.

(16) The gross appearance of the tissues at necropsy presents nothing that is characteristic. There is marked congestion of all viscera and there may be hemorrhages. Microscopic examination usually reveals cellular thrombi in the blood vessels. Perivascular lymphocytic infiltration is not found.

(17) There are no data to prove or disprove the efficacy of botulinus antitoxin as a prophylactic or therapeutic agent. In all recorded cases when homologus antitoxin has been administered it has been given too late in the course of the intoxication to be of any value.

(18) The diagnosis of botulism was in 11 outbreaks established by the demonstration of the toxin of *B. botulinus* in the remnants of the causative food. These tests have been very thorough in the recent outbreaks caused by ripe olives and spinach. In six instances the food was not tested for toxin, but *B. botulinus* was isolated in shake or enrichment cultures; in three outbreaks the bacteriological diagnosis was made from jars of home-canned vegetables of the same lot that had caused the poisoning.

In eight single or group outbreaks the remnants of the suspected food were not available and the diagnosis was made indirectly, namely, *B. botulinus* was isolated in five instances from the crop or gizzard of poisoned chickens, in two observations from the stools of clinical cases, and in one outbreak from the jejunal walls of a fatal case.

(19) Only 30 outbreaks or 32.9 per cent of the available observations have been proven bacteriologically. *B. botulinus*, type A, has been identified in 21, while type B has been found in 3 outbreaks.

(20) The epidemiologic data indicate that a true botulism infection following the ingestion of toxin free spores never occurs in human beings. In four instances it is proven that the eating of uncooked spoiled food caused botulism while the ingestion of portions of the same food after cooking provoked no symptoms. Canned goods deemed to be abnormal either by appearance of the container or by the odor should be destroyed, not heated and eaten.

(21) Spoilage of home or commercially canned food products due to *B. botulinus* represents an insignificant part of the yearly pack. It is the result of inadequate sterilization. The usual methods of processing must be systematically studied and the packer should aim at absolute sterility of the food products wherever it is possible.

Spoilage due to *B. botulinus* has been limited to a few definite varieties of plant products as home-canned string beans, corn, asparagus, spinach, also home-canned beets, apricots, and pears, and commercially pickled ripe olives, commercially packed spinach, string beans, and beets and corn. *B. botulinus* spoilage in ordinary commercially packed fruits and such vegetables as tomatoes has not been mentioned in the reports. Furthermore many vegetables such as corn and peas require such heavy processes to destroy the nontoxic thermophilic spoilage organisms found in them that the occurrence of botulism from them is very improbable.

(22) In the United States preserved plant products are primarily involved in botulism outbreaks while in Germany pork and fish products contain the fatal poison. In both countries botulism is caused by the use of preserved foods which are not thoroughly cooked before they are eaten.

PART X.

SUMMARY.

Since 1899 a total of 91 single or group outbreaks of botulism have been reported in the United States and Canada. A total of 345 people with 213 deaths or a case mortality rate of 61.7 per cent has been recorded. Only 30 outbreaks or 32.9 per cent of the available observations have been proven bacteriologically or toxicologically. The causative food consisted in 63 outbreaks (69 per cent, 25 proven, 38 not proven or inconclusive) of home or commercially canned plant products, while animal products have been found responsible for 19 (20.9 per cent, 5 proven and 14 not proven or inconclusive) single or group intoxications.

The following food products have been proven or assigned for the various cases of botulism: Home-canned string beans (17), home-canned corn (9), home-canned asparagus (5), home-canned apricots (3), home-canned pears (2), home-canned spinach (2), home-canned beets (1), home-brew liquor prepared from old home-canned products (1), home-prepared cottage cheese (2), home-preserved pickled mackerel and herring (1), home-cured ham (2), home-prepared blood sausage (1), home-cured salted pork (?), and home-prepared beef products (3); commercially packed spinach (6), commercially canned minced olive relish (3), commercially pickled and bottled ripe olives (7), commercially packed beets (2), commercially canned string beans (3), commercially canned corn (1), commercially canned pork and beans (1), commercially bottled tomato catsup (1), commercially prepared sausages (2), commercially cured ham (1), commercially bottled clam juice (2), commercially canned tuna fish (1), commercially canned evaporated milk (1), and commercially prepared minced chicken (1).

Spoilage due to *B. botulinus* can not always be determined by the appearance or odor of the food.

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AN EXPERIMENTAL STUDY OF THE METHODS AVAILABLE FOR THE ENRICHMENT, DEMONSTRATION AND ISOLATION OF B. BOTULINUS IN SPECIMENS OF SOIL AND ITS PRODUCTS, IN SUSPECTED FOOD, IN CLINICAL AND IN NECROPSY MATERIAL. I.*

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The bacteriology of botulism has, up to the present time, received scanty attention, because the investigation of the subject is fraught with technical difficulties and because the physiologic or experimental study of the disease offers more dramatic and obvious results. According to Burke,[1] 12 pure cultures of B. botulinus were available in 1919, in the United States; while, in 1921, Weiss,[2] in his study on the heat resistance of the spores of this organism, was able to use 14 to 16 toxin producing strains. In a recent communication Reddish[3] reported on 19 strains of B. botulinus, of which 18 were found to be contaminated with B. sporogenes. Bitter,[4] however, stated, in 1921, that in comparison to the fairly frequent occurrence of botulism in Europe, B. botulinus had been cultivated in a relatively small percentage of instances; namely, 12 isolations had been accomplished from food products, necropsy material or the feces of a hog. Most of these strains have been lost or, from the experience of one of the writers (K. F. M.), are so badly contaminated that they are useless for bacteriologic study. The conclusion of Bitter, that the demonstration and the isolation of B. botulinus from suspected food continues to be a comparatively difficult task, finds some justification in the fact that only 13, or 19%, of the 68 German botulism outbreaks have been bacteriologically proved. On the other hand, the studies of Randall,[5] Nevin,[6] Dickson,[7] Burke,[8] Thom, Edmonson and Giltner,[9] Armstrong, Story and Scott,[10] Graham[11] and others in this country furnish sufficient evidence that this anaerobe can be readily detected and purified from spoiled food material, provided suitable procedures and specimens are examined. Thirty-five, or

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Received for publication, July 26, 1922.

* This study was aided by grants from the National Canners Association, the Canners League of California and the California Olive Association.

[1] Jour. Bacteriol., 1919, 4, p. 555.

[2] Jour. Infect. Dis., 1921, 28, p. 70.

[3] Ibid., 1921, 29, p. 120.

[4] Ergebn. d. allg. Path. u. path. Anat., 1921, 19, II, p. 762.

[5] Med. Rec., 1920, 98, p. 763.

[6] Jour. Infect. Dis., 1921, 82, p. 226.

[7] Monograph of Rockefeller Institute, No. 8, 1918, p. 29; Jour. Am. Med. Assn., 1918, 71, p. 518.

[8] Jour. Bacteriol., 1919, 4, p. 541.

[9] Jour. Am. Med. Assn., 1919, 73, p. 907.

[10] Public Health Repts., 1919, 34, p. 2877.

[11] Jour. Bacteriol., 1919, 4, p. 1. McCaskey: Am. Jour. Med. Sc., 1919, 158, p. 57.

34.6%, of the 101 recorded outbreaks have been proved bacteriologically or toxicologically. These differences in the percentages of isolations reported for Europe and the United States are probably not due to the superior technic employed in the latter country, but to peculiarities in the food products causing the intoxications. The isolation from spoiled food is probably simplified on account of the enrichment of the toxicogenic microbe and the relative scarcity of other bacteria. This scarcity may be the result of the selective action of the heat employed in the preservation of the products. It is known that in Germony only 2, while in the United States 78, outbreaks were caused by food preserved by heat.

A perusal of the publications dealing with the attempts to isolate B. botulinus from vegetables, manure and soil specimens, which contained few spores of B. botulinus and a mixed flora consisting of sporulating aerobes and anaerobes, clearly demonstrates that the customary methods employed hitherto have proved inadequate for dealing with numbers of strains of the organism. This is illustrated by the failures of van Ermengem[12] and Bitter[13] to demonstrate B. botulinus in feces of animals and fishes and by the recent observations of Burke.[8] The latter worker made 235 cultures from specimens consisting of tap water, hay, leaves, vegetables, soil, etc.; in 11, or 5%, of the cultures the toxin of B. botulinus was demonstrated, but the anaerobe was only isolated in a pure state from 2 cultures.

The collection of a large number of strains of B. botulinus from diverse sources suitable for a comparative bacteriologic and serologic study could only be attempted when the demonstration and isolation of the organism from bacterial mixtures was attended with less difficulty. The study of the distribution and habitat of B. botulinus in nature could not be approached with any degree of confidence until a practical method of examination had been elaborated. Although the problem appeared rather complex, sufficient information was available from previous work and from the intensive studies on anaerobes conducted during and immediately after the war to suggest some methods of attack. It is the purpose of this communication to outline the experiments which led to the use of suitable enrichment cultures and to procedures which made possible the isolation of a fair percentage of pure strains of B. botulinus.

EXPERIMENTAL DATA

It is well known that B. botulinus can best be recognized and differentiated from other anaerobes by the action of its toxin on small laboratory animals. Repeated observations have shown that the poison may develop in mixtures, notwithstanding the presence of other aerobic or anaerobic bacteria. This latter property can therefore be used for the detection of B. botulinus. It is equally true that a medium encouraging the toxin production will also lead to an enrichment of B. botulinus.

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[12] Handb. d. pathogen. Mikroorganism. 1912, 4, p. 936.

[13] Reference 4, p. 791.

However, when the studies on which this paper is based were undertaken, the biochemical requirements of a culture fluid necessary to toxin production were poorly understood. In fact, some of the published statements were contradictory; a few workers preferred an alkaline reaction, while others selected a neutral culture fluid made of pork. The period of incubation, the temperature and oxygen requirements, etc., essential for the maximum toxin production had received some attention, but the published experiences were frequently not confirmed, and one gained the impression that many of the results depended more on chance than on reliable procedure. The experiments planned to elaborate a practical method for the enrichment and subsequent isolation of B. botulinus in pure culture had therefore to taken into consideration the following subjects: (1) composition and reaction of the culture mediums; (2) methods of anaerobiosis; (3) precautions against contaminations; (4) amount and preparation of field material to be examined; (5) period of incubation; (6) storage of cultures; (7) identification of toxin; (8) enrichment of weekly toxic cultures.

COMPOSITION AND REACTION OF CULTURE MEDIUMS

The original observations made by van Ermengem[14] that B. botulinus grew abundantly and produced a potent toxin in double strength, slightly alkaline meat infusion broth containing 1% peptone, 1% sodium chloride and 2% glucose have until recently been responsible for the use of this medium when selecting a substratum for the isolation of the organism from food or field specimens. Some workers adhered faithfully to the formula and employed a pork infusion broth of a reaction of 0.5% alkaline to phenolphthalein. It has already been pointed out that Dickson and Burke, Graham and others succeeded in isolating B. botulinus with the aid of this medium. The successes can probably be attributed to one or two causes: (1) cultures made of the samples contained a large number of spores of B. botulinus, and (2) a relatively small number of concomitant anaerobic spore bearers. Theoretically the medium of van Ermengem, although excellent for the toxin production with pure strains of B. botulinus, is, on account of its high carbohydrate content and relatively low buffer value, not selective for proteolytic anaerobes. Biochemical studies indicate that the van Ermengem broth favors the saccharolytic anaerobes and, when inoculated with a mixture of anaerobes, reaches frequently an H-ion concentration which is inhibitive for B. botulinus or at least for the enzymes assumed to be responsible for the liberation of the specific toxin. Furthermore, it has been noted that sporulation of B. botulinus is usually poor in van Ermengem mediums, as a result of which the isolation of this organism by selective heating of the enrichment culture is impossible. Chemical studies have shown that a pork base offers no advantages over a veal or beef infusion. In a search for a suitable medium, it was fully realized that solid mediums in the form of blood-agar plates or shake cultures in deep agar were useless on account of the comparative scarcity of B. botulinus spores in most of the specimens studied in the beginning of this investigation. The ideal medium for the isolation of B. botulinus would therefore be a fluid medium which favored the growth of that organism and restrained completely the development of the other anaerobic and aerobic bacteria commonly present in the soil and its products. The same considerations led Tulloch,[15] in 1917, to prepare a medium that provided enrichment of B. tetani in cultures made from wounds containing a variety of organisms. This worker noted that a substratum in which B. sporogenes had grown for some time was not suitable for the growth of the saccharolytic anaerobes or of B. sporogenes itself. Miss F. Field, of this laboratory tried, in tedious and time consuming experiments, to develop "exhaust mediums" suitable for B. botulinus. In these experiments, meat and vegetable substratums were inoculated with B. sporogenes, soil anaerobes, etc., and incubated for varying periods of time in open and closed vessels, but no medium thus prepared showed consistently selective properties for B. botulinus. Attempts were also made to enhance the selective properties of the exhaust mediums by the addition of pieces of string beans, glycerol, starch, etc., but the results were unsatisfactory. In fact, the observations collected in the course of these studies indicated that a completely exhausted medium for B. sporogenes could not be obtained and that a fluid medium partially inhibitive for B. sporogenes invariably exerted the same influence on B. botulinus. Furthermore, the preparation of exhaust mediums is time consuming and expensive when used for the enrichment of soil specimens weighing from 50 to 100 gm. These conclusions influence in no way the value of the exhaust medium of Tulloch for the isolation of B. tetani. A comparative study, conducted by one of us (K. F. M.), in 1918, confirmed the facts established by this worker. However, one must appreciate that the bacterial flora of a wound infected with the spores of the tetanus bacillus is less complex than that ordinarily encountered in specimens submitted to a search for B. botulinus. It is not unlikely that the addition of a piece of rabbit kidney enhances the growth promoting properties of the exhaust medium for B. tetani, which has already been selectively enriched in the wound secretion. The experience of this laboratory, however, indicates that the exhaust medium of Tulloch loses its selective property in the isolation of B. tetani from garden soil or other specimens containing a rich flora of anaerobic bacteria. Similar observations probably prompted Adamson [16] to recommend an alkaline broth, instead of an "exhaust medium" for the isolation of this particular organism.

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[14] Ztschr. f. Hyg. u. Infectionskrankh., 1897, 26, p. 1.

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[15] Jour. Roy. Army Med. Corps, London, 1917, 29, p. 631.

In the preliminary investigations, it was noted that the elaboration of toxin by impure cultures of B. botulinus was excellent in a beef heart peptic digest medium [17] employed for the studies of anaerobes in this laboratory. Subsequent biochemical studies to be published elsewhere have confirmed these observations and furnished accurate data explaining fully the superior properties of this medium over the others commonly used in the study of anaerobes. Furthermore, it was determined that the medium of van Ermengem could be improved by reducing the amount of glucose from 2 to 1%, by adding 0.5% dibasic potassium phosphate, only 0.5% sodium chloride and by adjusting the final reaction to P_H 8.4. This medium buffers excellently the volatile and fixed acids produced by the anaerobes; thus the H-ion concentration rarely increases over a P_H of 7 to 7.2. In fact, the reaction remains at the optimum H-ion concentration of the growth curve of B. botulinus, as will be shown by C. C. Dozier, in another paper. It is advisable either to add the necessary amount of glucose in the form of a 25%

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[16] Jour. Path. & Bacteriol., 1920, 23, p. 241.

[17] Preparation of beef heart peptic digest liver broth (for principles involved see Holman: Jour. Bacteriol., 1919, 4, p. 149; Stickel and Meyer: Jour. Infect. Dis., 1918, 23, p. 68, and H. H. Heller: Jour. Bacteriol., 1921, 6, p. 445). (1) Slowly heat to boiling finely ground, fat free heart, 1,000 gm., and tap water, 1,000 c.c.; adjust to a reaction of P_H 8.0-8.2, then cool and carefully skim off the layer of fat which floats on the cold medium. To each liter of beef heart mash, add 2 liters of peptic digest broth (see 2). Adjust the reaction to P_H 7.2-7.4. (2) Wash clean and mince finely 5 or more large pigs' stomachs. Mince an equal amount of clean pig's or beef liver. Mix in the following proportions:

Minced pigs' stomachs	400 gm.
Minced liver	400 gm.
Hydrochloric acid (Baker Chemical Co.)	40 gm.
Tap water at 50 C.	4,000 gm.

Keep the mixture in glass or porcelain receptacles for 18-24 hours. Make biuret and also tryptophan test. When both reactions are positive, the digest is green-yellowish and contains little undigested débris. Transfer to large bottles and steam for 10 minutes at 100 C. to stop digestion. Strain the digest through cotton, or preferably store over night in the ice chest and decant after 24 hours. Warm the decanted digest to 70 C. and neutralize with sodium carbonate (twice normal solution) to litmus at this temperature. Filter the desired amount, add 0.2% dibasic potassium phosphate; adjust to P_H 7.4 and mix with beef heart mash. Adjust the final reactions and sterilize for one hour at 18 lbs. of pressure. Incubate for 5 days and repeat the same sterilization for one hour at 18 lbs. of pressure.

solution after sterilization of the alkaline broth in the autoclave or to adjust the reaction of the medium with sterile sodium carbonate solution shortly before use. Live steam sterilization in an Arnold steam sterilizer produces less change and a medium of superior properties, but for reasons to be discussed later, this method of sterilization should not be employed for anaerobes. The beef peptic digest liver broth can be autoclaved without inducing changes in the reaction or in the growth supporting and enhancing properties of the enrichment mediums. The optimum reaction of this medium is a P_H of 7.0-7.4.

TABLE 1

Results of Experiments with Soil and Vegetable Specimens

Number	Material	Medium	Incubation, Days	Result
3 A	Soil 5 gm.	van Ermengem	22	Negative
3 B	Soil 5 gm.	Beef heart digest	22	2 c c toxic for guinea-pigs in less than 24 hours
3 C	Bean stalks 20 gm.	van Ermengem	22	Weak toxin, 2 c c toxic for guinea-pigs after 12 days
3 D	Bean stalks 20 gm.	Beef heart digest	22	2 c c toxic for guinea-pigs in less than 24 hours, type A
3 E	Bean casing	van Ermengem	22	2 c c toxic for guinea-pigs in 48 hours, type A
3 F	Bean casing	Beef heart	22	2 c c toxic for guinea-pigs in less than 24 hours, type A
10 II	Soil 10 gm.	van Ermengem	28	2 c c nontoxic
10 Ia	Soil 10 gm.	Beef heart digest	28	2 c c toxic for guinea-pigs in less than 24 hours, type A
10 Va	5 gm. decomposed hay	van Ermengem	28	2 c c nontoxic
10 Vb	5 gm. decomposed hay	Beef heart digest	28	2 c c toxic in 5 days
0K II	Turnips 20 gm.	van Ermengem	34	Nontoxic
0K II	Turnips 20 gm.	Beef heart digest	34	2 c c toxic for guinea-pigs in 5 days
0K III	Cherries 20 gm.	van Ermengem	34	Nontoxic
0K III	Cherries 20 gm.	Beef heart digest	34	2 c c toxic for guinea-pigs in 6 days
05K II	Leaf mold	van Ermengem	25	2 c c toxic for guinea-pigs in 5 days
05K II	Leaf mold	Beef heart digest	25	2 c c toxic for guinea-pigs in 3 days, type A
0K5 V	Asparagus tip	van Ermengem	34	2 c c toxic for guinea-pigs in 6 days
0K5 V	Asparagus tip	Beef heart digest	34	2 c c toxic for guinea-pigs in 2 days, type A
02K 9	Asparagus stalks	van Ermengem	38	2 c c nontoxic
02K 9	Asparagus stalks	Beef heart	38	2 c c toxic for guinea-pigs in less than 16 hours, type B

The value of the two mediums has been repeatedly tested: three experiments carried out early in the course of this investigation are, however, chosen for a discussion of the relative merit of the two mediums.

Exper. 1, 2 and 3.—Soil and vegetable specimens were suspended in salt solution heated for from 1 to 2 hours at 70 C. The heated suspensions were divided in equal portions and mixed in flasks with modified van Ermengem's broth (P_H 8.4) or beef heart peptic broth (P_H 7.4), exhausted and incubated for from 22 to 38 days. The results are summarized in table 1.

It is evident from the data presented in table 1 that the modified van Ermengem medium is decidedly inferior to the beef heart digest broth. Seven specimens contained a sufficiently large number of spores to produce in the latter medium a strong toxin, which could be identified by an antitoxin neutralization test, while only one glucose broth culture gave the same result. In fact, 6 cultures made in this medium were nontoxic and failed to indicate the presence of B. botulinus spores in the specimen. In 3 van Ermengem cultures the toxin produced was very weak and its nature could not be proved by the customary antitoxin test. Entirely similar results could be cited from other experiments.

Another great disadvantage of the van Ermengem broth is the violent gas production which takes place in soil cultures. A considerable number of culture flasks broke under the pressure of the liberated gases and valuable specimens were lost in this manner.

Repeated attempts to isolate B. botulinus in a pure state from positive enrichment cultures in van Ermengem's medium were either not successful or were in comparison with those made from beef heart mediums considerably more difficult. The principle of selective heating could not be applied to these cultures, probably on account of the absence of, or the low heat resistance of, the spores. These and other observations led to the routine use of a beef heart peptic digest liver broth for the enrichment and demonstration of B. botulinus.

METHODS OF ANAEROBIOSIS

Most of the descriptions of B. botulinus in the literature designate the organism as a strict anaerobe. It is stated that this bacterium can grow only in the presence of atmospheric oxygen, provided symbiotic organisms, as for example sarcina, cocci or B. subtilis, are present (van Ermengem,[18] Shippen[19] and others).

Studies conducted by F. Field and C. C. Dozier and numerous incidental observations made in this laboratory indicate that B. botulinus can probably grow in an environment which contains about ⅔ atmospheric oxygen. Loosely packed, damp hay or soil inoculated with detoxified spores will become toxic in the presence of air. The aerobic index determined for B. botulinus according to the procedure of Harris in a 2% glucose van Ermengem broth varies from 60/0 to 110/0. Pure and mixed cultures of B. botulinus grow abundantly in beef heart digest broth which is not stratified with petrolatum or freed from air by heating before inoculation. Toxins varying in potency from 10,000 to 50,000 M L D per c c for 250 gm. guinea-pigs inside of 96 hours following subcutaneous injection are regularly produced. It is frequently noted that the growth in beef heart cultures is more rapid and vigorous when the culture flasks are partially exhausted. The following explanation can be offered for this observation. The soil specimens are suspended in bottles with an equal part of salt solution; after heating for from 1 to 2 hours, the content is thoroughly mixed with freshly boiled, oil-stratified beef heart digest medium. In the course of shaking, the medium retains a considerable amount of air, which escapes slowly from the culture fluid. In this connection, it should be stated that the paraffin oil stratified on the medium is used to prevent undue evaporation of the fluid during the prolonged incubation, which is necessary to test the sterility of the broth. This oil usually forms an emulsion with the soil-beef heart material when shaken and increases the air content of the culture. For this reason, it has been found advantageous to mount a rubber stopper fitted with a bent glass tubing in the neck of the bottle and to exhaust the content with an electric pump. In order to procure a vacuum of from 5 to 15 mm., it is necessary to cover the stoppers and the bottle neck with "Imperial cement." The glass tubing leading to the pump is allowed to seal itself off by placing it in the middle of a small Bunsen flame. In order to possess comparative data, both the experiments with van Ermengem and beef heart digest medium must be carried out by this method. As already stated, the violent gas production in the glucose medium leads frequently to breakage of the bottles, and it has been found necessary to loosen the stoppers on the third or fourth day of incubation.

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[18] Reference 12, p. 922.

[19] Arch. Int. Med., 1919, 23, p. 388.

Since the beef heart digest medium has been used in a routine way for the enrichment of B. botulinus, attempts have repeatedly been made to replace the procedure of exhaustion by stratifying the medium with petrolatum. As a rule, exceedingly satisfactory results have been obtained, but it has been found necessary to add the melted petrolatum after the heated samples have been mixed with the medium. Some observations indicate that this method is not entirely free from dangers of contamination, and it has therefore been used only in exceptional instances. It must, however, be emphasized that the methods mentioned are needed only when mass cultures, using from 20 to 100 gm. of material, are used. Under ordinary circumstances, perfect enrichment and toxin production is obtained in test tubes containing freshly boiled beef heart digest mediums without petrolatum.

PRECAUTIONS AGAINST LABORATORY CONTAMINATIONS

The relatively frequent demonstration of B. botulinus in specimens of soil, vegetables, etc., was so startling and contrary to previous experiences that the question of contaminated culture mediums, glassware, etc., was seriously considered and accordingly investigated. In view of the precautions that have been taken to eliminate any possibility of cross contamination, this source of error can be absolutely excluded.

As early as 1908, T. Smith[20] called attention to the inefficiency of discontinuous steaming of culture mediums. He found that in the routine preparation of diphtheria toxin B. tetani survived the repeated steamings. Anybody working with anaerobes frequently makes the observation that some tubes of the pasty culture mediums, as for example brain or cooked beef heart mediums, may contain B. sporogenes after prolonged incubation. For technical reasons, it has been customary to sterilize these mediums by intermittent heating in the Arnold sterilizer. This procedure may be adequate for a laboratory in which little or no anaerobic spore material is prepared or investigated; it becomes, however, an unreliable method when hundreds of mass cultures, some containing billions of most resistant spores, are being handled in the course of a single day. If one, furthermore, appreciates that the spores of B. botulinus can survive 4 to 5 hours' boiling, and that the heated spores may frequently not germinate in from one week to several months, the potential danger of inadequate sterilization is quite evident. It is a common experience that lack of proper recognition of these dangers not infrequently leads to an extensive pollution of all the equipment of a cleaning kitchen and a culture medium department, and without proper sterilization a certain percentage of preparations will be found contaminated with the organism which is being studies in the laboratory. In the examination of specimens of soil and its products, additional danger exists in the dust produced in handling such material. In this connection it appears advisable to record one of the many experiments which have been made in this laboratory to determine the distribution of the spores of B. botulinus on the working benches, etc.

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[20] Jour. Am. Med. Assn., 1908, 50, p. 929.

Exper. 4 (*Series 146*).—The dust, etc., resting on various working benches, shelves, etc., was removed by means of sterile cotton pellets held by a sterile forceps. The cotton was placed in sterile salt and heated for 2 hours at 70 C., then mixed with 100 c c of sterile beef heart digest medium and treated according to the adopted procedure. The flasks were incubated for 10 days at 35 C. The results are presented in table 2.

It is evident from the data presented in table 2 that even in a laboratory in which the danger of dissemination of heat resistant spores has been fully recognized, some places are found to be contaminated. However, this and subsequent tests furnish definite assurance that the technic on the working benches on which field samples are handled is sufficiently rigid to prevent the contamination of field specimen cultures.

In order to assist workers who are confronted with a similar problem, the various methods employed are briefly summarized:

New and used glassware, discarded cultures in bottles or tubes, etc., are sterilized for 3 hours at 18 pounds' pressure. The sterilized glassware is cleaned by boiling in strong soda solution, dipped in dilute hydrochloric acid and then rinsed thoroughly in tap water. Dried and plugged test tubes, flasks, etc., are sterilized in the hot air sterilizer for 3 hours at 170 to 180 C. Culture mediums, salt solutions in sterilized containers, pipets, rubber stoppers, etc., are sterilized at 18 pounds' pressure for one hour. All mediums are incubated for from 5 to 14 days and then resterilized for 1 hour. Since the adoption of these precautions, nonsterile mediums have not been encountered. The working benches are covered daily with fresh, sterile wrapping paper which is soaked with a 10 to 15% dilution of compound solution of cresol. The same antiseptic is used for the washing down of shelves, tables, balances, etc. Pipets, mortars, etc., infected with cultures or soil are placed either in 50% dilution of compound solution of cresol or 20% formaldehyde solution. Tests conducted by C. C. Dozier have shown that the usual antiseptic solutions employed by bacteriologists for the destruction of pathogenic nonsporulating bacteria fail to destroy the spores of B. botulinus in less than 8 days. Weighing and bottling of soil or other field specimens is done in a large enamel tray (50 x 50 cm.) the bottom of which is covered by a layer of 25% dilution of compound solution of cresol. For special work, a sterile inoculation room is frequently used, but in the experience of this laboratory, the sterility of culture mediums and glassware is of greater importance than the precautions against air contaminations.

TABLE 2

Results of Experiment 4

Sample	Specimen Cultures	Result of Culture
1	Dust collected from table and bench on which soil samples are weighed and distributed in flasks	Nontoxic
2	Dust from shelves of medium cabinet........................	Nontoxic
3	Dust from shelves on which sterile glassware is stored......	Nontoxic
4	Working bench of A; at this place numerous samples of spore suspensions are counted daily	2 c c toxic for guinea-pigs in 4 days
5	Working bench of B; at this place mass cultures are prepared for serologic tests	2 c c toxic for guinea-pigs in less than 16 hours
6	Working bench of C; at this place mass cultures have been purified; hundreds of flasks have been inoculated with pure spore cultures of B. botulinus	Nontoxic
7	Working table used for necropsies, etc.	2 c c produced symptoms, but guinea-pig recovered
8	Wrapping paper used as covers on table and cabinet mentioned under (1) and (2)	Nontoxic
9	Dust from the floor of an incubator room....................	2 c c toxic for guinea-pig in less than 16 hours
10	Dust from shelves and tables of cleaning kitchen used for the storage of culture flasks, etc., previous to sterilization; 2 samples each	2 c c toxic for guinea-pig in less than 16 hours

Amount and Preparation of Field Material to Be Examined

Spoiled and toxic vegetable foods contain, as a rule, numerous viable spores of B. botulinus; enrichment methods are then unnecessary, and the organisms can frequently be isolated in deep agar shake cultures. Preliminary studies with soil specimens which as a result of enrichment cultures were known to contain spores of B. botulinus, failed to furnish the desired information relative to the number present in a weighed amount of earth. Numerous samples were cultivated by distributing from 1 to 10 gm. of heated soil by the dilution method either in deep agar shake tubes or in blood-glucose-agar plates, but toxin-producing colonies could not be isolated. It was therefore necessary to estimate the relative numerical distribution of the spores of B. botulinus by indirect methods. Theoretically, it was assumed that within certain limits the larger a sample examined the greater the probability of procuring toxic enrichment cultures. Furthermore, the use of specimens varying in amounts from 10 to 50 gm. partially eliminated as a factor of importance the well known uneven distribution of the spores in soil. These considerations were, however, of little value without a definite knowledge relative to the sensitiveness of the enrichment method. The question—"How many viable spores must be present in a soil sample to produce a toxic enrichment culture?"—was therefore submitted to an experimental study.

Heating of field specimens in order to eliminate nonsporulating aerobes and undesirable anaerobes is commonly used by the anaerobist, but deserves additional investigation so far as B. botulinus is concerned. Dickson and Burke[7] heated the contents of crops and gizzards or of the intestinal canal of poisoned chickens for one hour at 60 C. In a more recent publication, however, Burke[8] failed to state the method of preparation of the specimens she tested for the presence of B. botulinus spores. The impression is conveyed that the various samples of vegetables, hay, manure and soil were not subjected to heat.

The experiments dealing with the various phases considered in the foregoing are herewith detailed. Numerous quantitative tests using van Ermengem's medium and beef heart peptic digest liver broth have been carried out. Samples of soil or fruits were cultivated in amounts of 1, 10, 20 and 50 gm. Two experiments are presented to illustrate the character of the tests.

TABLE 3

Results in Exper. 5

Specimen	Amount of Chopped Olives, Gm.	Culture Mediums	
		van Ermengem	Beef Heart Peptic Digest Broth
Unbroken olives	10.0	Nontoxic	Toxic; type B isolated
From tree	1.0	....	Nontoxic
Bird pecked olives	10.0	Toxic; weak toxin	Toxic; type B isolated
	1.0	....	Toxic; weak toxin
Olives buried in soil	10.0	Nontoxic	Toxic; weak toxin
	1.0	....	Nontoxic

Exper. 5 (Ser. 4).—Three lots of olives collected from a ranch in Northern California were chopped up separately, and heated for one hour at 65 C. Ten and 1 gm. lots were cultivated in 100 c c amounts of van Ermengem's and beef heart digest broth. The cultures were incubated at 38 C. for 30 days. The results are shown in table 3.

The superior properties of the beef heart broth as an enrichment medium are well illustrated in this experiment. Specimens which give toxic cultures are either nontoxic in van Ermengem's broth, or the toxin is not sufficiently potent for proper identification. The number of viable spores after heating for one hour at 65 C. is small in the unbroken and bird pecked olives, inasmuch as 1 gm. samples furnish only weakly toxic or nontoxic cultures.

Exper. 6 (Ser. 122 B).—Virgin soil collected from a recent rockslide in Yosemite Valley was distributed in 1, 10, 20 and 50 gm. lots in bottles containing 10 c c of salt solution. The suspensions were heated for 2 hours at 70 C., then mixed with 100 c c of beef heart medium and incubated for 10 days at 35 C. The 1 gm. cultures were nontoxic, while the 10, 20 and 50 gm. series produced toxins which were fatal in 2 c c amounts to guinea-pigs in less than 16 hours. B. botulinus, type A, was isolated from several cultures.

From these and similar experiments, it appears advisable to recommend the cultivation of fairly large vegetable and soil specimens, unless the material is relatively rich in other saccharolytic and proteolytic anaerobes. This reservation is prompted by the observation that, whereas small quantities (10 gm.) of manured soil furnish toxic cultures, 50 or 100 gm. specimens reveal no demonstrable toxin when treated under identical conditions. An explanation for these differences will be attempted later in the discussion on the deterioration of the toxin in anaerobic cultures.

TABLE 4

Results in Exper. 7

Number of Bottle	Soil	Number of Spores and Rods Inoculated	Result
1	10 gm. (39. II-V)	6 spores and 3 rods	2 c c of culture toxic for guinea-pig in 24 hours
2	10 gm. (39. II-V)	13 spores and 3 rods	2 c c of culture toxic for guinea-pigs in 24 hours
3	10 gm. (39. II-V)	5 spores and 3 rods	Nontoxic
(4)	10 gm. (39. II-V)	10 spores and no rods	2 c c produced symptoms and death in 17 days

A series of tests has been carried out in order to determine the approximate number of spores which must be present in specimens of soil, hay, etc., in order to produce a toxic enrichment culture.

The question: "How many spores inoculated into culture mediums containing heated soil will germinate and produce toxin in the presence of other bacteria?" was approached with the aid of Barber's single cell isolation method. Two types of experiments can be reported.

Exper. 7.—Five bottles containing 100 c c of beef heart peptic digest broth and 10 gm. of heated mixed soil (2 hours at 70 degrees), which on repeated tests was found free from B. botulinus, were inoculated with a few unheated spores and vegetative rods of B. botulinus, strain 38, picked by the Barber method from a 10-day culture in plain gelatine. The cultures were kept at 35 C. for 10 days. The results are summarized in table 4.

Exper. 8.—The experiment was similar to that reported under Exper. 7; however, the soil and the spores (strain 62) picked under the microscope were heated simultaneously for 2 hours at 70 C. The suspension was then mixed with the beef heart digest broth, exhausted and incubated for 10 days at

37 C. The spores of B. botulinus were subjected to the same treatment as was customary in the testing of field specimens. The results are shown in table 5.

Unheated spores added to heated soil emulsions in beef heart peptic digest broth are capable of germination and multiplication. It is, however, impossible to obtain identical results by repeating the same procedure; only 2 or 3 of the 10 flasks inoculated with the same number of spores and rods may give toxic cultures. Moreover, these experiments are not a duplicate of the method employed for the enrichment of

TABLE 5

RESULTS IN EXPER. 8

Number of Bottle	Soil	Number of Spores	Result
1	5 gm. of (84 c. VIII)	2	2 c c produced symptoms and death on the 16th day
(2)	5 gm. of (84 c. VIII)	3	Nontoxic
(3)	5 gm. of (84 c. VIII)	4	Nontoxic
(4)	5 gm. of (84 c. VIII)	7	Nontoxic
(5)	5 gm. of (84 c. VIII)	9	Nontoxic

TABLE 6

RESULTS IN EXPER. 9

Series		20 Gm. Soil	Medium	Number of Spores Added Previous to Heating	Result After Incubation for 10 Days at 37 C.	Result After Incubation for 10 Days at 28 C.
A	1	Nonsterile	Beef heart digest	1,000 to 10,000	2 c c toxic in 16 hours	Nontoxic
	2	Sterile	Beef heart digest	1,000 to 10,000	2 c c toxic in 16 hours	2 c c toxic in 16 hours
	3	Sterile	Peptic digest no meat	1,000 to 10,000	2 c c toxic in 16 hours	2 c c toxic in 16 hours
B	4	Nonsterile	Beef heart digest	100 to 1,000	2 c c toxic in 48 hours	Nontoxic
	5	Sterile	Beef heart digest	100 to 1,000	2 c c toxic in 16 hours	2 c c toxic in 16 hours
	6	Sterile	Peptic digest	100 to 1,000	2 c c toxic in 16 hours	2 c c toxic in 16 hours
C	7	Nonsterile	Beef heart digest	10 to 100	2 c c toxic in 5 days	Nontoxic
	8	Sterile	Beef heart digest	10 to 100	Toxic in 24 hours	Toxic in 16 hours
	9	Sterile	Peptic digest	10 to 100	Toxic in 16 hours	Toxic in 16 hours
D	10	Nonsterile	Beef digest	1 to 10	Nontoxic	Nontoxic
	11	Sterile	Beef digest	1 to 10	Toxic in 16 hours	Nontoxic
	12	Sterile	Peptic digest	1 to 10	Toxic in 16 hours	Nontoxic

field specimens and therefore are of little value to test the sensitiveness of the culture medium, etc. Nevertheless, they indicate definitely that a few viable spores accidentally introduced as contaminations may produce toxic cultures and furnish misleading results. In the light of the observations made in exper. 7, the elaborate precautions against contaminations appear fully justified.

TABLE 7

RESULTS IN EXPER. 11

Series		Soil, Gm.	Medium	Number of Spores Added Previous to Heating	Result After 10 Days' Incubation Subcutaneous Injection
A	1	50	Beef heart digest broth	10,000 to 100,000	1 c c toxic in 24 hours
	2	50	Beef heart digest broth	10,000 to 100,000	1 c c toxic in 30 hours
	3	50	van Ermengem mod.	10,000 to 100,000	1 c c toxic in 48 hours
	4	50	van Ermengem mod.	10,000 to 100,000	1 c c toxic in 30 hours
B	5	50	Beef heart digest broth	1,000 to 10,000	1 c c toxic in less than 16 hours recovered organism
	6	50	Beef heart digest broth	1,000 to 10,000	1 c c toxic in 40 hours
	7	50	van Ermengem mod.	1,000 to 10,000	1 c c toxic in 48 hours
	8	50	van Ermengem mod.	1,000 to 10,000	1 c c toxic in 30 hours
C	9	50	Beef heart digest broth	100 to 1,000	Nontoxic
	10	50	Beef heart digest broth	100 to 1,000	2 c c toxic in 60 hours
	11	50	van Ermengem mod.	100 to 1,000	Nontoxic
	12	50	van Ermengem mod.	100 to 1,000	Nontoxic
D	13	50	Beef heart digest broth	10 to 100	Symptoms, died 18th day
	14	50	Beef heart digest broth	10 to 100	2 c c toxic in 48 hours
	15	50	van Ermengem mod.	10 to 100	Nontoxic
	16	50	van Ermengem mod.	10 to 100	Nontoxic
E	17	50	Beef heart digest broth	No spores	2 c c nontoxic
	18	50	Beef heart digest broth	No spores	2 c c nontoxic
	19	50	van Ermengem mod.	No spores	2 c c nontoxic
	20	50	van Ermengem mod.	No spores	2 c c nontoxic
Controls		4 tubes each	Beef heart digest broth	10,000 to 100,000	All toxic, heated and unheated
		4 tubes each	Beef heart digest broth	1,000 to 10,000	All toxic, heated and unheated
		4 tubes each	Beef heart digest broth	100 to 1,000	All toxic, heated and unheated
		4 tubes each	Beef heart digest broth	10 to 100	Heated: 2 toxic, 2 nontoxic; unheated: 3 toxic, 1 nontoxic

A few spores added to soil suspensions and subsequently heated in the customary manner give nontoxic or weakly toxic cultures. Frequent repetitions of exper. 8 with as many as 40 spores fail to produce toxic cultures. These observations are confirmed by the following experiments.

Exper. 9.—Twenty gm. of soil repeatedly found free from spores of B. botulinus were suspended in salt solution. A mixture of spores produced in gelatine broth (strains 58 and 62) were counted in a Helber chamber; dilutions of the spore suspension were made in salt solution and 1 c c each of the various dilutions were added to the nonsterile and sterile soil. The suspensions were heated for one hour at 70 C., mixed with beef heart digest broth and exhausted. One series of flasks was incubated at 35 C., while the other was kept at 28 C. for 10 days. The results are shown in table 6.

Exper. 10.—The same experiment was repeated with the same soil specimens and with spores of strain 90. The same results were obtained as in exper 9.

Exper 11.—A mixture of 8 different soil specimens, free from spores of B. botulinus was distributed in 50 gm. amounts in a series of bottles and then suspended in salt solution with a P_H of 7.4. Four bottles received 1 c c each of varying dilutions of an unheated spore suspension of B. botulinus strain 97 (140 hours old). The dilutions were prepared with great care and were controlled by deep agar shake cultures. The soil-spore suspensions were heated for one hour at 70 C., mixed with either beef heart digest or van Ermengem medium and incubated for 10 days at 35 C. The results are presented in table 7.

TABLE 8

RESULTS IN EXPER. 12

Series	20 Gm. Soil	Medium	Number of Spores Added Previous to Heating	Result After 10 Days' Incubation
(1)	Nonsterile	Beef heart peptic digest	400 to 600	Toxic in less than 16 hours
(2)	Sterile	Beef heart peptic digest	400 to 600	Toxic in less than 16 hours
(3)	Nonsterile	Beef heart peptic digest	40 to 60	Nontoxic
(4)	Sterile	Beef heart peptic digest	40 to 60	2 c c produced symptoms but guinea-pig recovered

Exper. 12.—The number of spores added to the soil suspensions was in this experiment determined by the plating of heated and unheated spore dilutions in blood agar. Spores (strains 90 and 47) which were dried for 24 hours on sand over sulphuric acid were used. The treatment of the inoculated soil suspensions was similar to that employed in the preceding experiments. The results are shown in table 8.

The data presented in exper. 9, 10, 11 and 12 conclusively indicate that toxic soil enrichment cultures are regularly obtained, provided the specimens contain at least from 100 to 1,000 viable heat resistant spores. Exceptions may occur, as, for example, in exper. 11, ser. C and D, one sample containing less than 100 spores proved sufficiently toxic to kill a guinea-pig in 48 hours, while of those containing from 100 to 1,000 spores, only one furnished a toxic enrichment culture. It is obvious that in order to demonstrate the existence of B. botulinus spores in soil specimens, adequate samples of not less than 10 gm. should be chosen. When the question is sufficiently important, it is recommended to prepare a series of cultures containing 1, 10, 20, 50 and 100 gm. of soil.

The heating of soil, vegetable or food specimens previous to making of cultures is a recognized practice among workers with sporulating anaerobes. The methods which have proved satisfactory in this laboratory have recently been reported by H. H. Heller.[21] Concerning B. botulinus, nothing definite was known when these studies were begun, but by analogy it was assumed that toxic enrichment cultures could probably be procured more readily after destroying the nonsporulating bacteria. Rabinowitsch,[22] for example, reports that the heating of specimens used for the isolation of B. tetani is very important. Thus, toxic cultures only were procured when the 2% glucose broth in which 1 lb. of strawberries had been washed was heated for 30 minutes at 80 C. In judging the advantages and disadvantages of heated specimens for enrichment cultures, it appears advisable to consider the heat resistance of the spores of B. botulinus. In the American literature the conception previals that the spores produced in various culture mediums are very resistant to heat, while in Europe the original observations of van Ermengem, who found the spores to be readily killed after an exposure to 80 C. for 30 minutes, are assumed to be correct. It must be remembered, however, that the germination of the spores may be considerably retarded when exposed to heat and that the resistance of the prevailing spores, as found in nature, is unknown. In view of these mentioned conditions, it is suspected that frequently nontoxic enrichment cultures, and consequently the studies dealing with the distribution of the spores of B. botulinus, furnish an erroneous conception of actual conditions. Experiments to be reported indicate that it is technically impossible to prove this point. Thus, unheated soil specimens, containing viable spores of B. botulinus in small numbers, furnish nontoxic, or weakly toxic, enrichment cultures. The various concomitant aerobes or anaerobes apparently overgrow B. botulinus, or the enzymes of these bacteria destroy the generated toxin.

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[21] Jour. Bacteriol., 1921, 6, p. 445.

[22] Arch. f. Hyg., 1907, 61, p. 103.

From a practical standpoint, it appears important to know the special distribution of those spores which are very resistant to heat. It is this type which, at least in this country, causes the majority of botulism outbreaks. A number of experiments have been carried out to investigate this question. Two are described in detail to illustrate the methods and results.

Exper. 13.—A mixture of 6 different soil specimens previously found to be free from spores of B. botulinus was distributed in 20 gm. amounts into a series of bottles and suspended in salt solution. Two bottles received 1 c c each of varying dilutions of an unheated spore suspension of B. botulinus 97. As controls, bottles containing salt solution were inoculated with the same suspension; one soil-spore and the control salt solution spore suspension culture were heated for one hour at 70 C. The unheated and heated bottles were filled with beef heart digest medium, exhausted and incubated for 10 days at 35 C. The results are shown in table 9.

TABLE 9

Results in Exper. 13

Series		Treatment Previous to Making of Cultures	Number of Spores Added	Result After 10 Days' Incubation at 37 C.
A	1	Not heated	1,000 to 10,000 in soil	2 c c produced symptoms but guinea-pig recovered
	2	Heated at 70 C.	1,000 to 10,000 in soil	2 c c toxic in 18 hours
	3	Heated at 70 C.	1,000 to 10,000 in salt solution	2 c c toxic in 18 hours
B	1	Not heated	100 to 1,000 in soil	2 c c toxic in 5 days
	2	Heated at 70 C.	100 to 1,000 in soil	2 c c toxic in 5 days
	3	Heated at 70 C.	100 to 1,000 in salt solution	2 c c toxic in 18 hours
C	1	Not heated	10 to 100 in soil	Nontoxic
	2	Heated at 70 C.	10 to 100 in soil	2 c c toxic in 16 days
	3	Heated at 70 C.	10 to 100 in salt solution	2 c c toxic in 18 hours
D	1	Not heated	1 to 10 in soil	2 c c nontoxic
	2	Heated	1 to 10 in soil	2 c c nontoxic
	3	Heated	1 to 10 in salt solution	2 c c toxic in 48 hours
E	1	Not heated	No spores	Nontoxic
	2	Heated	No spores	Nontoxic

Exper. 14.—The intestinal washings of one human case of botulism were distributed into 10 bottles each containing 75 c c. A formed stool specimen was carefully emulsified and also divided into 6 portions of 50 c c each. One-half of the bottles of each series of stool specimens was heated for 1½ hours at 60 C., mixed with beef heart digest broth, exhausted and incubated at 37 C. for 10 days. The results of this experiment are shown in table 10.

From the results obtained in expers. 13 and 14, one may conclude that soil and stool specimens must be heated previously in order to obtain toxic enrichment cultures. The outcome of these tests is so conclusive that further comment is unnecessary and heating of such specimens for 2 hours at a temperature of from 60 to 70 C., or one hour at 80 C., must be made a routine practice.

It should be stated in this connection that very acid food samples, etc., should be heated only after complete neutralization. The procedure suitable for this purpose will be considered in subsequent paragraphs of this paper.

TABLE 10

Results in Exper. 14

Specimen	Treatment Previous to Making of Cultures	Result After 10 Days' Incubation at 37 C.
Intestinal washing 1	Heated	2 c c toxic in 24 hours } Isolated B. botulinus type A
2	Heated	2 c c toxic in 18 hours
3, 4, 5, 6	Heated	2 c c nontoxic
Intestinal washing 7, 8, 9, 10, 11, 12	Not heated	All nontoxic
Formed stool 1	Heated	2 c c toxic in 18 hours, B. botulinus type A
2, 3	Heated	Nontoxic
Formed stool 4, 5, 6	Not heated	All nontoxic

PERIOD OF INCUBATION

Two questions deserve experimental investigation, namely: 1. What is the optimum temperature for the growth of B. botulinus in mixed cultures? 2. What period of incubation at a temperature of 28 to 37 C. furnishes toxins of the highest potency?

It is well known that van Ermengem,[14] Römer,[23] Würcker[24] and other European bacteriologists described strains of B. botulinus which developed quickly and abundantly at a temperature of from 20 to 30 C. Landman[25] and later Leuchs[26] noted, however, that one strain of B. botulinus isolated from string beans (Darmstadt outbreak) grew very well at 37 C., although the toxin production was better at 24 C. In a recent compilation, Bitter[4] concludes that all the strains of B. botulinus known in Germany until 1919 may grow in artificial culture mediums at a temperature of 37 C., but that the microbe produces the most potent toxin when incubated at from 25 to 30 C. Up to three or four years ago these views have guided the workers in the United States, and the early studies made on B. botulinus were conducted with cultures which were kept at 28 C. The publications of Nevin,[6] Shippen,[19] Thom, Edmonson and Giltner,[9] Orr[27] and of Burke,[1] which report typical growth and excellent toxin production at 37 C. with American strains of B. botulinus isolated from a variety of food and soil products, were available when the studies presented in this paper were undertaken. As far as enrichment cultures with soil, hay and vegetable specimens is concerned, it should be stated that Burke[8] adopted an incubation period of from 2 to 6 months at 28 C. This worker, however, states that "this incubation period was too long, as the average life of B. botulinus in broth cultures is about three months." A higher percentage of positive cultures could have been obtained, in the opinion of Burke, provided the cultures had been incubated for only 6 to 10 weeks at 28 C. Quite recently, Kendall[28] recommended that B. botulinus should be grown at 30 instead of at 38 C.

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[23] Centralbl. f. Bakteriol., Orig. 1900, 27, p. 857.

[24] Sitzungsber. d. physikal-medizin. Sozietät in Erlangen, 1909, 41, p. 209.

[25] Hyg. Rundschau, 1904, 14, p. 449.

[26] Ztschr. f. Hyg. u. Infectionskrankh., 1910, 65, p. 55.

[27] Jour. Infect. Dis., 1922, 30, p. 118.

It is evident from this review that the information regarding the optimum temperature conditions for the growth of B. botulinus in enrichment cultures is incomplete and contradictory. The knowledge relative to the incubation period required for the production of a toxin of highest potency is equally unsatisfactory. For example, Thom, Edmonson and Giltner[9] usually obtained a toxin with a potency of 0.001 c c for guinea-pigs in cultures incubated for 6 days at 37 C.; while the best toxin (M L D 0.0001 c c) was produced at 35 C. for 28 days. Orr[29] tested toxins which had been generated in cultures kept for 3 weeks at 37 C.; the M L D for mice did not exceed 0.0001 c c. Systematic studies to determine the optimum incubation period for B. botulinus in a suitable culture medium have not been carried out. In fact, the first hundred enrichment cultures prepared in this laboratory were incubated empirically for 4 weeks at 37 C. It was, however, noted that specimens which were originally toxic, containing B. botulinus in large numbers, would produce weakly toxic enrichment cultures. Theoretically such cultures could only result from (1) deterioration of the toxin at body temperature, or (2) destruction of the toxin molecule by the concomitant bacteria present in the enrichment culture. A systematic study of this question was therefore undertaken by a number of workers in this laboratory. The findings which have a direct bearing on the problem under consideration are briefly mentioned:

A comparative study of over 100 strains of B. botulinus from various sources has shown that growth and toxin production is best at a temperature of 35 C. According to thermographic records, the incubation rooms installed in this laboratory have an average temperature of 35 C. Pure cultures of B. botulinus in beef heart digest medium produce on the 10th to 12th day of incubation at 35 C. toxins of the maximum potency; the M L D (96 hours) for a guinea-pig of 250 to

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[28] Ibid., 1921, 29, p. 231.

[29] Jour. Med. Res., 1921, 42, p. 127.

300 gm. is usually from 0.00002 c c to 0.0001 c c. Incubation at the same temperature for 15, 20, 25, 30, etc., days leads to a distinct deterioration of the toxin. These studies will be reported in a series of papers, dealing with the biochemical activities of B. botulinus in various mediums. The behavior of the toxin in enrichment cultures is similar to that in pure cultures; as a rule, the deterioration is more rapid, as will be noted in exper. 15 and 16.

Exper. 15.—A series of enrichment cultures which proved very toxic on the 10th day were kept in the incubator for 30 days. Additional toxin tests were made on the 20th and 30th day, and the data are presented in table 11.

TABLE 11

Results in Exper. 15

Number of Sample	Nature of Specimens	1st Test 10th Day at 35 C.	2d Test 20th Day at 35 C.	3d Test 30th Day at 35 C.	Type of B. botulinus
2 1 c. M.	Soil	2 c c toxic in 16 hours	2 c c toxic in 24 hours	2 c c toxic in 36 hours	A
40 M. M.	Soil	2 c c toxic in 24 hours	2 c c toxic in 6 days	2 c c nontoxic	A
202 V	Soil	2 c c toxic in 16 hours	2 c c toxic in 48 hours	2 c c toxic in 12 days	A
203 XIII	Beanstalk	2 c c toxic in 16 hours	2 c c toxic in 48 hours	2 c c toxic in 5 days	B
205 XIV	Soil	2 c c toxic in 16 hours	2 c c toxic in 5 days	2 c c nontoxic	A
207 XII	Soil	2 c c toxic in 16 hours	2 c c toxic in 16 hours	2 c c toxic in 24 hours	B
207 XII	Soil	2 c c toxic in 16 hours	2 c c toxic in 3 days	2 c c toxic in 6 days	B
209 XXIII	Soil	2 c c toxic in 48 hours	2 c c toxic in 10 days	2 c c nontoxic	A
209 XX	Cucumber	2 c c toxic in 16 hours	2 c c toxic in 24 hours	2 c c toxic in 3 days	A
214 40	Soil, England	2 c c toxic in 40 hours	2 c c toxic in 5 days	2 c c nontoxic	B
214 60	Soil, England	2 c c toxic in 16 hours	2 c c toxic in 24 hours	2 c c toxic in 24 hours	B
217 III	Soil	2 c c toxic in 16 hours	2 c c toxic in 4 days	2 c c toxic in 15 days	A

Exper. 16.—The filtered toxin of strain 38 (M L D ± 0.0001) was divided into two large test tubes, 75 c c each. To tube 1, were added 5 c c of the sediment of a 10 days' soil culture which was nontoxic and free from B. botulinus, but contained various aerobes and anaerobes; and to tube 2, 5 c c of a carefully sterilized soil suspension were added. Both tubes were incubated at 35 C., and the toxin content determined on the 5th and 15th days. The results are shown in table 12.

The findings in exper. 15 and 16 indicate that the toxin of B. botulinus deteriorates when kept at 35 C. The rate of destruction is not influenced entirely by the concomitant bacteria of the enrichment culture, but probably depends on the degree of toxicity reached on the 10th day. In other words, a highly toxic enrichment culture kept near body temperature may remain fatal for guinea-pigs, while weakly toxic cultures become nontoxic during the same period of incubation at the same temperature. It naturally suggested itself to study the influence on toxicity of incubation at 28 C. A number of tests similar to those reported under exper. 10 were carried out. Without going into details, it was noted that frequently nontoxic cultures were obtained at 28 C., even after prolonged observation, although incubation at 35 C. produced satisfactory specimens. Lack of proper incubation room facilities made it unpractical, however, to handle many hundreds of cultures at a temperature of 28 C., although the observations were sufficiently numerous to warrant a positive deduction.

TABLE 12

RESULTS IN EXPER. 16

Tube	M L D at Beginning	5th Day at 35 C.	15th Day at 35 C.
Toxin + soil culture	0.0001 c c	0.01 c c (growth)	0.6 c c
Toxin + sterile soil	0.0001 c c	0.01 c c (sterile)	0.6 c c

TABLE 13

RESULTS IN EXPER. 17

Specimens	Time Required for 2 c c of the Centrifugalized Supernatant Fluid of a Culture to Kill Guinea-Pigs of 300-350 Gm.			
	24 Hours	48 Hours	5 Days	10 Days
1 gm. of soil	Nontoxic	Nontoxic	Nontoxic	Nontoxic
10 gm. of soil	Nontoxic	Nontoxic	Symptoms	4 days = 1 M L D
20 gm. of soil	Nontoxic	48 hours	24 hours	16 hours
50 gm. of soil	Symptoms	48 hours	24 hours	16 hours

In order to prove that the most potent toxin is produced on the 10th day of incubation at 35 C., cultures were made from a number of virgin soil samples, which were known to contain spores of B. botulinus. Toxin determinations were made at varying times during the incubation. One of these experiments is herewith detailed.

Exper. 17.—Virgin soil from Yosemite Valley was distributed in bottles suspended in salt solution and heated for 2 hours at 70 C. The suspensions were mixed with peptic digest broth, exhausted and incubated at 35 C. The presence of toxins was determined by subcutaneous inoculations of guinea-pigs. The results are shown in table 13.

The evidence justifies the conclusion that an incubation period of 10 days at 35 to 37 C. in beef heart peptic digest broth furnishes the optimum conditions for securing highly toxic enrichment cultures.

Observations to be recorded in subsequent paragraphs also indicate that such cultures are well suited for the isolation and purification of B. botulinus from soil specimens.

STORAGE OF CULTURES

The examination of a large series of cultures, limited assistance, continuous observation of the inoculated guinea-pigs and occasionally a shortage of these animals necessitated a prolonged storage of the enrichment cultures. As each toxic culture was repeatedly tested and typed with antitoxins, it was essential to possess a practical method which would preserve the toxicity of the cultures at its original titer. The facts presented in the preceding paragraphs and the observations made on numerous cultures suggested storage at a low temperature. It was repeatedly noted that bottles containing a strong toxin would become nontoxic or weakly toxic and unsuitable for identification tests when kept during the summer months in a warm corner of the laboratory (about 30 C.). Facilities to store hundreds of bottles in the refrigerator were not available, but storage in dark cabinets at an average room temperature of 18 to 22 C. was practical. The value of this precedure has been tested and one of the experiments is herewith detailed.

Exper. 18.—Several enrichment cultures were stored under different conditions and the potency of the toxins estimated on guinea-pigs by the usual procedures. The results can be presented as follows:

Specimen Group 1: Originally 2 c c toxic for 250 gm. guinea-pig in 48 hours; at 18 to 22 C. for 20 days killed in 16 hours.

Specimen Group 2: Originally 2 c c toxic for 250 gm. guinea-pig in 48 hours; at 2 to 5 C. for 20 days killed in 40 hours.

Specimen Group 3: Originally 2 c c toxic for 250 gm. guinea-pig in 48 hours; at 35 C. for 20 days produced only symptoms, and the guinea-pig recovered.

Based on this and similar observations, it was concluded that the toxicity of an enrichment culture does not decrease; in fact it may increase when kept at a temperature of 18 to 22 C. Storage at this temperature has therefore been adopted as a routine procedure.

IDENTIFICATION OF THE TOXIN

In order to segregate the toxic from the nontoxic cultures, the following method was chosen: Ten c c of the supernatant fluid of the culture were removed, care being exercised to avoid stirring up the meat and soil sediment. These samples were centrifugalized for one hour at high speed (3,000-4,000 revolutions). One to 2 c c of the perfectly clear culture fluid were inoculated subcutaneously on the median abdominal line of a guinea-pig weighing not more than 400 gm. In case the animals succumbed to an intoxication[30] without exhibiting the symptoms and necropsy findings of other anaerobic infections, as "malignant edema" or tetanus, the culture was considered suspicious and the nature of the poison was determined by an antitoxin neutralization test. In 90% of the tests it was possible to eliminate the nontoxic samples by this procedure. The centrifugalized cultures were only filtered when other pathogenic anaerobic bacteria, as B. oedematiens, vibrion septique produced misleading symptoms and lesions. In some instances, the centrifugalized cultures were fed instead of injected. This procedure was adopted for the following reasons: 1. Filtration of large series of cultures is exceedingly time consuming and expensive. The Mandler candles on the market are of inferior quality and necessitate frequent replacement. 2. A considerable portion of the toxin is absorbed by the filter and weakly toxic cultures become unsuitable for toxin-antitoxin tests. 3. Feeding of large series of guinea-pigs is a tedious process and exceedingly inaccurate. In the majority of instances, the animals refuse to swallow or regurgitate the foul smelling culture fluid. 4. Subcutaneous injections allow quantitative tests and furnish additional information with regard to the distribution of B. tetani and vibrion septique. Intraperitoneal inoculations, on the other hand, frequently produce shock and sudden death, probably as a result of the various biogenous amines, etc., present in the cultures. Weakly toxic cultures are only detected following the injection of at least 2 c c. and therefore mice are found unsuitable and more expensive.

TABLE 14

RESULTS OF EXPERIMENTS TO ESTIMATE APPROXIMATE STRENGTH OF TOXINS IN ENRICHMENT CULTURES

Specimen	2 c c Killed 250 Gm. Guinea-Pig	Minimal Lethal Dose for 250 Gm. Guinea-Pigs Within 96 Hours, C c
158 soil type A	<16 hours	0.0002
156 stool type A	±28 hours	0.005
158/7 soil type A	+16 hours	0.0001
200 a III soil type B	+16 hours	0.0001
219 liver necropsy type A	<18 hours	0.00005
220/2 soil type B	48 hours	0.0005
220/10 soil type A	24 hours	0.01
221 A string beans type A	18 hours	0.00005
221 B string beans type A	18 hours	0.0001 (48 hours)
Exper. 11. A. L.	±30 hours	0.01
Exper. 11. A. 2	±24 hours	0.005

In order to estimate the approximate strength of the toxins present in enrichment cultures, a series of minimal lethal dose determinations was carried out. A few of the results are presented in table 14.

The results presented in table 14 indicate that soil enrichment cultures may produce exceedingly potent toxins. Cultures which are fatal to guinea-pigs in 2 c c amounts in less than 4 days can be used for identification tests. Furthermore, several hundred observations have demonstrated that only those cultures which kill in less than 18 hours are suitable for the isolation of B. botulinus in a pure state. Toxins producing symptoms in 3 to 4 days leading to death on the 5th to 8th day or even later have been classified as "weak toxins." They cannot be typed, but their significance and the methods employed in preparing such specimens for identification will be treated in the next article.

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[30] The necropsy findings on guinea-pigs which succumb to the toxin of B. botulinus are briefly as follows: A slight infiltration or edema, with or without hemorrhages, or frequently no macroscopic changes at the site of inoculation; marked congestion of the abdominal viscera with dark colored liver and spleen; in very acute intoxications these lesions are absent. Frequently a distended bladder and a rather dry appearance of the serous linings. Lungs always hyperemic and may show large or small pleural suggillations or petechiae.

The toxin identification tests were usually conducted on guinea-pigs; a limited number of cultures were also tested by intraperitoneal injections on mice. The antitoxins used were all monovalent type A and B horse or goat serums, with an antitoxin content of from 10 to 200 units per c c.[31] For routine tests, three animals were inoculated subcutaneously or intraperitoneally in the following manner:

TABLE 15

PROCEDURES FOR TOXIN IDENTIFICATION TESTS

Animal Number	Supernatant, Centrifugalized or Filtered Culture Fluid	Antitoxin
Guinea-pig 1	1 to 2 c c subcutaneously	
Guinea-pig 2	1 to 2 c c subcutaneously	0.5 to 1 c c of antitoxin for B. botulinus type A
Guinea-pig 3	1 to 2 c c subcutaneously	0.5 to 1 c c of antitoxin for B. botulinus type B
Mouse 1	0.1 c c intraperitoneally	
Mouse 2	0.1 c c intraperitoneally	0.5 c c of antitoxin type A
Mouse 3	0.1 c c intraperitoneally	0.5 c c of antitoxin type B

The toxins were mixed with the antitoxin in small test tubes and the mixtures were kept at room temperature for from one-half to 3 hours, depending on the number of samples. The toxin-antitoxin tests have been conclusive with cultures containing at least 1 to 10 minimal lethal doses per 2 c c. In the majority of experiments, the control animals inoculated with the toxin alone succumbed a few hours earlier than those inoculated with the toxin and nonspecific antitoxin. It should be emphasized that inconsistent neutralization tests were invariably repeated either with filtered toxins or by feeding the latter to animals which had received subcutaneously 0.5 to 1.0 c c of antitoxin. A limited number of toxins have been encountered[32] in which all 3 animals succumbed with symptoms of botulism or, if surviving, apparently incompletely protected guinea-pigs showed symptoms of intoxication. When this occurred, the experiment was repeated by inoculating a series of 5 animals. The first 3 were treated as shown in table 15, while the fourth received, besides the toxin, 1 c c each of antitoxin for B. botulinus type A and type B (1 c c of polyvalent antitoxin was used if available). The fifth guinea-pig received from 1 to 2 c c of filtrate or culture fluid which had previously been boiled for 10 minutes. The presence of a mixture of type A and B toxin and the presence or absence of heat resistant poison was demonstrated by this procedure. That this may be responsible for apparently equivocal results has been further proved by the isolation of B. botulinus type A and B from 2 different soil enrichment cultures.

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[31] Determined according to the method of Bengtson.

[32] The observations made in the laboratory have independently been confirmed by George E. Coleman of Santa Barbara.

Centrifugalized cultures producing tetanus were filtered; one observation had shown that this disease followed the inoculation of tetanus spores which had not been sedimented in the course of the centrifugalization. Some filtrates provoked symptoms of tetanus, and the test for the presence or absence of B. botulinus toxin was accomplished by mixing the filtrates with 1,000 units of commercial tetanus antitoxin or by feeding the suspected cultures to properly immunized guinea-pigs. A few toxic cultures could not be identified by the antitoxin-neutralization or feeding tests. Experiments are now in progress to determine the relationship of these toxic cultures to the recently described anaerobe, isolated by Miss Bengtson from the larvae of Lucilia Caesar.[33]

In conclusion, it must be emphasized that toxic enrichment cultures should be considered positive for B. botulinus only when a decisive toxin—antitoxin reaction is obtained, or when the enrichment cultures provoke classical intoxications on feeding, or when the specific organism is isolated in a pure state.

ENRICHMENT OF WEAKLY TOXIC CULTURES

It has been pointed out that enrichment cultures may frequently contain less than one minimum lethal dose per 2 c c. The inoculated guinea-pigs may exhibit for several days characteristic symptoms of botulism in the form of flabby abdominal muscles, striking emaciation, etc., and finally either recover or succumb to secondary infections. In the latter instance, the fairly characteristic necropsy findings are masked by a diplococcus, a B. bronchisepticus pneumonia or a generalized paratyphoid infection. At the beginning of the investigation an effort was made to produce a stronger toxin, by transplanting various amounts of the unheated or heated original enrichment cultures into flasks containing 100 c c of fresh sterile beef heart digest mediums and incubating again for 10 days at 35 C. This procedure had been used by Burke [8] with some success, but has not proved satisfactory in this laboratory. Transplants from cultures in van Ermengem mediums were invariably nonpoisonous, while the toxicity of beef heart cultures was only slightly enhanced over the original culture. Various modifications of heating the original culture at 80 to 100 C. were tried, but the toxicity was not appreciably increased. In fact, it was found advisable to prepare fresh cultures with the original soil or vegetable samples. This method has distinct advantages, namely: 1. It serves as a control for the original test. 2. It may yield at times more toxic cultures than the previous examination, which can be identified by an antitoxin—toxin test. 3. It may, furthermore, furnish cultures excellently suited for the isolation and purification of B. botulinus where this was not formerly possible.

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[33] Two series of experiments have shown that the toxin of the anaerobe isolated by Bengtson is produced in enrichment cultures and in the presence of soil bacteria. It is therefore not unlikely that toxin of some of the enrichment cultures which could not be neutralized by a polyvalent B. botulinus antitoxin belong to the group of the Lucilia Caesar poisons. This point can only be decided when a suitable antitoxin against the latter organism is available.

These tests, which were conducted at monthly intervals, served as sterility controls for the laboratory procedures, assured the workers that laboratory contaminations had been eliminated and provided data which indicated that the spores of B. botulinus were unevenly distributed in the specimens. These statements are best illustrated by the presentation of one test.

TABLE 16

FINDINGS IN EXPER. 19

Number	Specimens	1st Culture: 2 c c	2d Culture: 2 c c
(1)	Beets, Kansas (149)	Symptoms, death 48 hours	Death, 48 hours, type A
(2)	Soil, cloverfield	Death on 5th day paratyphoid infection	Nontoxic
(3)	Soil, cornfield	Death in 16 hours	Death, 16 hours, type B
(4)	Soja beans	Symptoms, recovered	Symptoms, death 12th day
(5)	Beet tops, Louisiana	Symptoms	Death 4th day, type A
(6)	Moldy hay	Nontoxic	Nontoxic
(7)	Rice straw	Symptoms	Death, 48 hours, type A
(8)	Soil, Pennsylvania	Symptoms	Nontoxic
(9)	Soil, pasture	Symptoms	Death, 18 hours, type A
(10)	Soil, ryefield	Symptoms	Death, 48 hours, type B
(11)	Garden soil	Death, 5th day	Death, 48 hours, type A
(12)	Soil, horse pen	Death, vibrion septique infection	Nontoxic
(13)	Soils, San Bernardino	Symptoms	Death, 48 hours, type B
(14)	Soil, Pasadena	Death in 72 hours	Marked symptoms
(15)	Dirt, horse corral	Nontoxic	Nontoxic
(16)	Soil, hog pen	Death, 4th day	Death, 48 hours, type B
(17)	Decayed alfalfa	Death in 24 hours, pneumonia	Nontoxic
(18)	Virgin soil	Death in 36 hours, vibrion septique	Nontoxic
(19)	Soil, Alleghany mountains	Death in 36 hours, tetanus	Nontoxic
(20)	Potato pits, Yakima	Death in 24 hours, type A	Nontoxic

Exper. 19.—Specimens which produced unsatisfactory enrichment cultures on account of prolonged incubation or storage and those which were frankly nontoxic were repeatedly cultivated in beef heart digest broth and incubated for 10 days at 35 C. The findings are presented in table 16.

The value of making repeated cultures on doubtful specimens is fully established by the data shown in table 16. It is unnecessary to state that several hundreds of identical tests have been carried out with similar results. Occasionally, as shown under No. 20, a specimen produces an original toxic culture, but on retesting it is found to be nontoxic. Cultures of such specimens have been made a third and fourth time, and in this manner evidence is procured which indicates that heat resistant spores may be quite unevenly distributed in soil or vegetable specimens. In order to prove the presence or absence of B. botulinus spores, repeated cultures should be regularly made from the original specimens, and doubtful cultures should be tested by this procedure instead of transplanting enriched cultures to fresh mediums.

Based on the experiments described in the foregoing paragraphs, the following method is now used for the enrichment and demonstration of B. botulinus in specimens of soil and its products:

THE METHOD EMPLOYED IN THIS LABORATORY

Twenty to 50 gm. of soil or vegetables or fruit peelings, etc., are placed in a 250 c c culture flask (No. 2221, Arthur H. Thomas Catalogue) and thoroughly suspended or mixed in sterile salt solution (P_H 7.0). Moist soil is previously ground in a mortar. Hay and dry vegetable specimens are finely chopped and then soaked for several hours in salt solution. The suspensions. etc., are heated in a water bath for 1 to 2 hours at 60 to 70 C. or 1 hour at 80 C. While warm, the suspensions are mixed with 100 c c freshly boiled beef heart peptic digest liver broth with a reaction of P_H 7.2-7.4. The fluid is either covered with petrolatum or liquid petrolatum to avoid evaporation, or the flasks are closed by a sterile rubber stopper provided with a piece of glass tubing. The container is rapidly exhausted and the glass tubing is allowed to seal itself off by placing it in the middle of a small bunsen flame. The cultures are incubated for 10 days at 35 C. A small sample of the supernatant fluid is then removed and centrifugalized for 1 hour at high speed. Two c c of the clear fluid are injected subcutaneously along the median line of the abdomen of guinea-pigs weighing 250 to 300 gm. The symptoms of the animals are recorded by daily inspections, and the dead guinea-pigs are carefully necropsied. Cultures which are fatal to guinea-pigs are further tested by a toxin-antitoxin test with type A and B antiserums. When the cultures contain less than one M L D per 2 c c, cultures of the original material are made a second, even a third and fourth time. Centrifugalized cultures are only filtered through small Mandler candles provided the inoculated guinea-pigs succumb to mixed anaerobic infections or present symptoms of tetanus. Such fluids may be tested by feeding immunized and nonimmunized guinea-pigs with heated and unheated cultures. The latter procedure is, however, less dependable than subcutaneous inoculation. Filtered or centrifugalized cultures which are known to be free from concomitant anaerobes may be "typed" on mice by intraperitoneal inoculations. Specimens are considered positive for B. botulinus only when a decisive antitoxin-toxin test has been obtained or the organism has been isolated in pure culture.

In order to avoid laboratory contaminations, the culture mediums, salt solution, flasks, etc., should be autoclaved at 18 pounds' pressure for 1 hour and resterilized for the same period after an incubation of from 5 to 14 days. Contaminated glassware, cultures, etc., are treated similarly for 3 hours at

18 pounds' pressure. The working benches, trays, balances, etc., are cleansed with 50% dilution of a compound solution of cresol or warm 10 to 20% formaldehyde solution. Inexperienced workers should question their results until they have eliminated the danger of laboratory contaminations by continuous, painstaking vigilance and repeated control examinations.

THE ISOLATION AND PURIFICATION OF B. BOTULINUS FROM TOXIC SOIL ENRICHMENT CULTURES

It has been pointed out that highly toxic cultures may be obtained by proper enrichment methods, although it is impossible to demonstrate B. botulinus by the customary procedures employed for the direct primary isolation of anaerobes. The statement made by a group of workers that they "never failed to obtain the organism in pure culture from the broth in which a virulent toxin had developed," applies in all probability to the enrichment cultures obtained from spoiled food in which B. botulinus was the main and often the only spore-bearing anaerobe.

In the course of a number of preliminary tests during which small amounts of toxic enrichment cultures were distributed by dilutions into deep liver agar shake tubes, it was noted that only those samples which were fatal to guinea-pigs in less than 16 hours (2 c c subcutaneously) were of sufficient promise to devote time and material for isolation. This process of selection reduced the specimens suitable for isolation to less than 10% of the cultures, but even the agar columns of these samples rarely presented colonies in sufficient numbers or of characteristic structure to permit of their rapid and conclusive identification as those of B. botulinus. These failures led to a number of experiments with selective mediums. Attempts were made to enrich overwhelmingly the B. botulinus present in the original enrichment culture by successive passage through mediums of various composition and reaction. Invariably, a substratum favoring B. botulinus encouraged also the associated proteolytic anaerobes. Certain suggestive leads have been encountered in the course of these studies, and it is not unlikely that a selective medium may be developed in the future. As it was, however, imperative to isolate in the shortest possible time a large series of strains of B. botulinus, it was found necessary to make use of the exceptionally marked heat resistance of the spores of B. botulinus. Two procedures have yielded a number of successful isolations, namely:

1. The enrichment cultures (10 days at 35 C.) are either heated at 80 C. for 1 hour or boiled for 10 minutes. The sediment of the cultures is sealed in 1 to 2 c c amounts in small glass tubes and subjected to the heat. Small

samples of the heated specimens are immediately plated in sheep blood agar, or sown into deep liver agar tubes or enriched for from 24 to 120 hours in beef heart peptic digest broth and then cultivated in deep agar. The latter procedure may be repeated leading frequently to enrichments suitable for isolations.

2. Twenty to 50 gm. of soil, which had previously been found to contain a large number of B. botulinus, are suspended in an equal amount of salt solution. The suspensions are exposed to steam in an Arnold sterilizer for from 1 to 1½ hours. The heated soil specimens are then cultivated in beef heart peptic digest broth according to the customary procedures. Subcultures are made daily in deep agar shake tubes. Several strains have been isolated by this method; in the heated cultures B. botulinus and occasionally B. sporogenes and B. welchii are the only surviving and freely growing anaerobes.

As a part of the practical control of botulism in the United States rests primarily on an accurate knowledge of the maximum resistance of the spores to heat, the use of these procedures appears justified. It may be assumed that the boiling or steaming selectively eliminated the nonresistant spores and in this manner furnished strains of maximum resistance. For studies dealing exclusively with the biology and the distribution of various types of B. botulinus in nature these methods are, therefore, not practical. It has, for example, been impossible to obtain toxic enrichment cultures by boiling or steaming soil samples which contained the spores of B. botulinus type B. Furthermore, many interesting problems dealing with the distribution of the various mutants of the genus Botulinus cannot be solved by "heating methods," and, therefore, no effort should be spared to develop a selective enrichment medium. It is self evident that the procedures mentioned can also be applied to the isolation of B. botulinus from the intestinal contents and the decomposed viscera of man and animals. Subsequent paragraphs will consider the details to be observed in handling these specimens.

One of the writers (K.F.M.) has used, since 1907, the deep agar shake method for the primary isolation and purification of anaerobes. Various modifications and improvements have recently been summarized in several papers by H. H. Heller.[21] The technical principles mentioned in these communications have been followed in the isolation of B. botulinus and need no additional comment. Peptic digest or veal infusion glucose (0.5%) agar has frequently been used for primary isolation instead of liver agar. In searching the agar column of the cultures for characteristic colonies, a Mayer's binocular dissecting microscope has been employed and found invaluable in saving time and eyesight. In this connection, it should be stated that only the mass colonies which correspond in general appearance to those described by Burke were considered characteristic. Experiences with over 100 cultures of B. botulinus isolated from various sources, which will be reported in another paper, indicate that the shape and the thickness of the colonies is sufficiently constant to enable detection in crowded mixed cultures. It is customary to use a fairly dry 2% agar and to incubate the various seed and dilution tubes at 28 to 35 C. for at least 10 days. The heating injures the spores considerably and delayed germination is frequently observed.

Laboratory workers who intend to isolate B. botulinus from mixed enrichment cultures should thoroughly familiarize themselves with the appearance of the mass colonies of authentic, recently isolated strains. In particular, the shape, size and texture of such colonies should be studied in deep agar of various composition and colloid content. This recommendation is made because certain toxic and pure stock cultures, circulating in this country, produce in deep agar atypical mutation colonies. These strains of B. botulinus convey an entirely wrong impression of the colony characteristics and, when adopted as a standard, prevent the inexperienced from selecting and fishing B. botulinus colonies. It is naturally quite possible that in this laboratory a number of toxic strains of B. botulinus have not been recognized and isolated on account of the adopted conservative policy of picking only disk-shaped colonies. In this connection, it must be admitted that even the latter type is not absolutely specific for B. botulinus. A number of anaerobes forming disk colonies not unlike those of B. botulinus have been found in soil enrichment cultures. These bacteria, when isolated and purified, resemble, morphologically and biochemically, B. botulinus, but they fail to produce a toxin. Experiments with these strains will be reported elsewhere more in detail. Another condition deserves attention: Crowded, deep agar shake tubes frequently reveal small, rather transparent disk colonies with heavy polar tufts. These colonies are usually formed by various terminal end spore bearers and are easily mistaken by the beginner for those of B. botulinus. It can be said without fear of contradiction that considerable experience is needed to be thoroughly familiar with the various types of disk colonies characteristic of B. botulinus.

The colonies which are considered typical are picked by means of fine capillary tubes and are transferred to a series of deep agar or beef heart peptic digest broth tubes. As soon as toxin-antitoxin tests on mice and microscopic examinations have proved the nature of the isolated colony and confirmed the type of the original enrichment culture, the process of purification is undertaken. Aerobic contaminations have usually been eliminated in the course of repeated heatings so that cultivation of young beef heart peptic digest broth cultures in deep agar tubes furnishes pure cultures in 95% of the instances. Cultures under inspection for purity are kept in beef heart broth and veal infusion digest broth for from 4 to 6 weeks at 28, 37 and 56 C. Shake cultures in deep agar are made at regular intervals; isolated colonies are picked and transferred to beef heart tubes. During the last year successive plating in sheep blood peptic digest agar with the aid of an anaerobic jar method to be described by C. C. Dozier, or according to the inverted plate method of Krumwiede and Pratt, or surface plating on milk agar, has been used to verify the purifications accomplished by the deep culture method. Several cultures have been further purified by Barber's microscopic method of single bacillus isolation. The employment of the latter procedure is, however, unnecessary. Several hundred different anaerobic cultures have been isolated and purified in this laboratory by the deep culture method, and it has been noted that the contaminations of properly purified cultures were either aerobes, as molds, cocci and subtilis types or adventitious anaerobes due to improperly sterilized mediums. The rigid examination of carefully purified cultures of B. botulinus during the last 2½ years has not shaken the original conclusion that the deep culture method, occasionally combined with the blood agar or milk agar plating method, is the most practical and reliable means for the isolation and purification of these organisms.

It is customary in this laboratory to preserve one culture of the original strain of anaerobe after purification in sealed tubes of beef heart peptic digest broth. In case of accidental contamination of the stock culture or for future reference these tubes saved time, worry and labor.

In conclusion, it should be emphasized that the primary isolation of B. botulinus from toxic soil, vegetable or manure enrichment cultures is only possible when the original specimen contains a fairly large number of heat resistant spores. Based on numerous observations, it is recommended that in enrichment cultures containing on the 10th day of incubation at 35 C. approximately 1,000 to 10,000 M L D isolation of the organism in pure culture should be attempted by the deep agar method. The results when the degree of toxicity is below this point are too generally negative to warrant the procedure.

EXAMINATION OF SUSPECTED FOOD

Before discussing the methods adapted to the examination of suspected food, it is deemed advisable to consider the principles involved and to indicate the great importance of this phase of technical mycology. The demonstration of B. botulinus and its toxin in preserved or canned vegetables, meats, etc., may have a twofold purpose, namely: 1. It may confirm a provisional clinical diagnosis and from a medicolegal standpoint explain the true nature of the intoxication, or (2) it may supply important information relative to the type of B. botulinus antitoxin which may be useful in the treatment of the disease. It is evident that in every suspected outbreak of botulism a toxicologic examination by inoculation or by feeding of the suspected food to guinea-pigs and mice should be carried out, and no effort should be spared to procure specimens suitable for this purpose. Little accurate knowledge relative to the average toxicity of spoiled canned foods is available. Future toxin tests should carefully determine the average number of lethal doses per 1 c c of vegetable juice. In case a specimen is nontoxic, cultures should be made. Occasionally the results may be vitiated by the fact that suspected foods are collected after an exposure to air and light for several days. Furthermore, that portion of the vegetables available for examination may have been subjected to more heat in the course of preparation than that consumed by the patients, or the sample may have been made susceptible in transit to factors which destroyed the toxin. Under these circumstances, what is apparently a nontoxic specimen will often furnish toxic enrichment cultures, or numerous typical colonies will develop in specimens prepared by the deep agar method.

Old and recent observations have shown that the development of B. botulinus and the generation of its toxin in home and commercially canned vegetables and fruits may be very irregular. It has been pointed out in another paper that the occurrence of demonstrable toxin in canned products is the exception rather than the rule. For example, Koser, Edmonson and Giltner[34] have shown that the contents of only 6 of 82 defective containers of spinach were found to be toxic when fed to guinea-pigs, while workers in this laboratory made similar observations on home canned string beans and asparagus. The content of one jar may be toxic, while 10 or 12 others packed at the same time and under identical conditions may be spoiled and nontoxic, or may be normal in appearance, and yet on enrichment may furnish a culture of B. botulinus. The present day information relative to the ability of B. botulinus to survive or to germinate in vegetables and fruits is incomplete, and a systematic search for this organism and its toxin in home and commercially canned food by direct or enrichment cultures appears imperative for the following reasons:

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[34] Jour. Am. Med. Assn., 1921, 77, p. 1250.

1. Nothing is known in regard to the distribution of B. botulinus spores in under-processed canned foods, whether spoiled or sound. The cultural methods employed by Weinzirl,[35] Cheyney,[36] and the Micro-biological Laboratory[37] of the Bureau of Chemistry are inadequate. The samples chosen for their tests were too small and no special anaerobic methods or mediums were employed. Until the possibility of botulinus spoilage had been recognized, processing procedures were frequently used which did not destroy the spores of B. botulinus. In the majority of instances the few surviving spores were seriously injured and germination failed to take place, or it was retarded for months. Larger samples of suspected food material, or cans which present no evidence of bacterial activity must, therefore, be enriched in suitable anaerobic mediums. These recommendations apply to commercially as well as to home canned foods; particularly in the latter products absolute sterility is usually impossible on account of the customary processing with boiling water. It would, therefore, be of great value to know accurately the average percentage of containers in households which hold viable spores of B. botulinus. With such information available, the factors preventing or enhancing the germination and toxin production could be studied more intelligently and the question, Why is botulism due to canned vegetables, etc., an acquisition of recent years? could probably be answered by reference to scientific facts instead of assumptions.

2. Direct cultures by the deep and plate agar methods should be made of toxic and nontoxic spoiled canned vegetables and fruits in order to determine the bacterial flora. By this procedure, the relative proportion of B. botulinus to other anaerobes or aerobes could be perfectly estimated and the influence of the latter organisms on the toxin production be established more accurately than has hitherto been attempted. Furthermore, these tests could serve as valuable contributions to the study of anaerobic spoilage occurring in canned products

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[35] Jour. Med. Res., 1919, 39, p. 349.

[36] Ibid., 1919, 40, p. 177.

[37] Jour. Am. Med. Assn., 1920, 74, p. 1220. Also reference 34.

and would either support or refute the contention of some workers who attribute great practical importance to the constant association of other bacteria with botulinus organisms. Numerous observations made in this laboratory have shown that commercially packed spoiled and toxic spinach contains no other organism than B. botulinus, and symbiosis is obviously not a prerequisite for toxin production in this vegetable. However, it has been fully proved that any lot of canned food which shows an appreciable percentage of spoilage is potentially dangerous, but no laboratory has as yet determined the percentage of containers of such lots which may harbor viable spores of B. botulinus. Until such tests have been carried out every can or jar of a batch or lot showing even less than 1% spoilage must theoretically be considered suspicious. It has been definitely proved that the presence of B. botulinus in canned food is always indicative of poor sterilization, and a few recent investigations have disclosed that not one container, but usually the entire lot or pack, has been improperly processed.

It will be evident from this brief discussion that the bacteriologic examination of suspected food, whether spoiled or sound, necessitates the use of proper procedures. The methods which have been found dependable for this purpose are, therefore, presented in detail.

A. Tests for Toxicity of Foods.—1. A 10 c c sample or the entire available amount of vegetable juice or brine is either centrifugalized or is filtered through paper and then centrifugalized. In some cases, the clarified specimens may still be contaminated with aerobic cocci and rods; filtration through a Mandler candle is then recommended. A portion of the filtrate or centrifugalizate is boiled for 30 minutes. Two mice each are inoculated intraperitoneally with 0.5 c c of unheated or boiled juice. Guinea-pigs weighing not more than 300 gm. are similarly treated with amounts of from 1.0 to 2.0 c c. The animals inoculated with the unheated material will succumb in from 4 to 72 hours, provided the food specimen contained the toxin of B. botulinus in sufficient concentration to produce symptoms or death in human beings, when ingested. It is self-evident that the animals injected with the heated specimens should remain alive, provided the specimen contains only the toxin of B. botulinus and no metallic poisons. For confirmation of the inoculation tests, it has frequently been found practical to feed samples of the suspected food to immunized and nonimmunized guinea-pigs.

2. In case of meat or vegetable foods, which contain little fluid, three procedures may be followed, namely: (a) A portion of the specimen may be administered by forced feeding to guinea-pigs, or (b) mixed with the regular feed offered these animals, or (c) a fairly large sample or the entire available remnant of the food may be ground in a mortar and then extracted for from one to two hours at room temperature with sterile salt solution. The extract is centrifugalized and the supernatant fluid is tested either directly or after filtration by the inoculation of mice and guinea-pigs, as outlined under (1), while the sediment is preserved for subsequent tests to be outlined under B.

3. Unopened, but suspected, tins or glass containers are thoroughly scrubbed with soap in hot running water, the top and sides dipped in a warm 20% formaldehyde solution and the latter permitted to act for from 10 to 15 minutes. The top or lids are flamed just before being opened. A "radial" can opener sterilized in oil or in the autoclave is used, cutting a circular opening of from 2 to 3 inches in diameter. A 10 to 15 c c portion of the juice or brine is pipetted into centrifuge tubes; with the aid of forceps, which have been sterilized in liquid paraffin oil of a temperature of 180 C., specimens varying from 25 to 250 gm. are transferred to sterile Blake bottles for extraction or cultures. The opening of the container is covered with sterile paper and the can stored in the icebox. The unheated and heated centrifugalized liquid portion or extract of the food is tested on mice or guinea-pigs as stated under (1).

4. The type of B. botulinus toxin present in foods is determined by the inoculation of 3 guinea-pigs or 3 mice, one with the centrifugalized or filtered juice or extract alone, one previously inoculated with from 0.5 to 1.0 c c type A antitoxin and another with type B antitoxin. In order to approximate the correct dose of toxin, serial inoculations of 3 mice each, as recommended by Bengtson[38] are sometimes necessary. The inoculations can be made as shown in table 17.

TABLE 17

SERIAL INOCULATIONS OF MICE TO DETERMINE CORRECT DOSE OF TOXIN

A	Mouse 1—1 c c of juice, brine or extract
	Mouse 2—1 c c of juice, brine or extract and 0.5 to 1.0 c c of type A antitoxin
	Mouse 3—1 c c of juice, brine or extract and 0.5 to 1.0 c c of type B antitoxin
B	Mouse 4—0.5 c c of juice, brine or extract
	Mouse 5—0.5 c c of juice, brine or extract and 0.5 to 1.0 c c of type A antitoxin
	Mouse 6—0.5 c c of juice, brine or extract and 0.5 to 1.0 c c of type B antitoxin
C	Mouse 7—0.1 c c of juice, brine or extract
	Mouse 8—0.1 c c of juice, brine or extract and 0.5 to 1.0 c c of type A antitoxin
	Mouse 9—0.1 c c of juice, brine or extract and 0.5 to 1.0 c c of type B antitoxin

The examination of feeds involved in outbreaks of fowl botulism or "forage poisoning" in horses has been conducted according to the same principles. Large samples of the feed, varying from 2 to 20 lbs. are soaked in sterile tap water for from 24 to 48 hours at room temperature. The fluid is recovered from the plant material by means of a meat press, strained through paper and tested either unfiltered by feeding or filtered by subcutaneous inoculations on guinea-pigs. "Mass enrichment" cultures are also made with the soaked and heated feed samples. As the workers of this laboratory have thus far been unable to detect the toxin of B. botulinus in moldy hay, grain or silage suspected of causing botulism in horses or cattle, it is impossible to conclude that the methods employed are really dependable.

B. Isolation of B. botulinus from Suspected Food.—Two procedures should be used: (1) deep agar shake cultures, and (2) enrichment "mass cultures" with heated and unheated specimens.

(1) The sediment of the centrifugalized juice or extract is divided into 2 portions, and 1 is heated in a thin, narrow tube for from 30 to 60 minutes at 70 C. Adequate samples of the heated and unheated specimens are distributed in melted liver peptone or peptic digest agar. It is advisable to prepare triplicate sets and to incubate one at 35 C., one at from 25 to 28 C. and

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[38] Public Health Reports, 1921, 36, p. 1665.

one at 55 C. This method permits a quantitative estimation of the number of viable organisms or spores per 1 c c of juice or brine. By making dilutions in broth and transferring 1 c c amounts to from 10 to 15 c c of melted agar in deep tubes or to warm blood agar tubes, which are poured into plates, isolated colonies are usually obtained. The inoculated tubes or plates should be kept under observation for at least 10 to 20 days. Characteristic disk-shaped colonies are readily counted and frequently permit the isolation of a pure culture.

(2) The sediment used for shake cultures or a 20 to 250 gm. sample or the entire available remnant of the food is suspended in appropriate amounts, heated for from 1 to 2 hours at 60 to 70 C. and then mixed with large amounts of freshly boiled beef heart peptic digest liver broth. In case the specimen is very acid or distinctly alkaline, as for example string bean salad prepared with vinegar or olive brine, it is advisable to neutralize the salt suspension with sodium carbonate or dilute lactic acid. Observations have shown that the heating of acid or alkaline specimens either destroys or injures the viable spores, and nontoxic enrichment cultures are sometimes obtained. Unheated specimens should also be tried in case of unsatisfactory nontoxic cultures.

The enrichment cultures are incubated for from 5 to 10 days at 35 C. and then tested for B. botulinus toxin. The principles followed for the isolation of B. botulinus from toxic cultures is the same as described in previous chapters. Purified cultures are identified by toxin-antitoxin tests. It is self-evident that the type of the isolated strain should correspond with that previously established for the toxin of the suspected food, juice or extract.

In order to stimulate a further investigation of suspected foods, either commercially packed or home canned products, etc., the observations made in this laboratory with the foregoing methods during the last two years are summarized in table 18.

EXAMINATION OF CLINICAL MATERIAL

In a previous communication,[39] the isolation of B. botulinus from the stools of patients with clinical cases has been described. Fecal specimens from 4 clinical cases (Reports 3, 4, and 63) obtained from 3 different outbreaks contained B. botulinus type A on the 6th, 7th, 11th, and 12th day, respectively, after the consumption of the causative meal. One specimen procured from a case on the 31st day (Report 63) and another from a case on the 26th day of illness (Report 81) were negative. Over 80 stool specimens of people who had ingested raw vegetables and fruits purchased in the open market proved negative for B. botulinus, even though such foods had demonstrated on repeated examinations the presence of botulinus spores.

The method employed is briefly as follows:

Stool specimens as large as possible are diluted with salt solution until the formed portions are finely divided. The emulsions are placed in 250 c c culture flasks and heated for one hour at from 60 to 70 C. They are then

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[39] Ibid., 1921, 36, p. 1313.

TABLE 18

RESULTS WITH PRESERVED FOODS

Number of Sample	Food or Food Products	Origin	Condition of Container	Physical Examination of Product		Toxin Test	Cultural Tests		B. botulinus
				Odor	Appearance		Aerobes	Anaerobes	
53 I a	Pickled ripe olives	L, California	"Swell"	Normal	Soft and discolored turbid brine	Negative	Spore bearing and gram-negative cocci	B. botulinus in enrichment also terminal end spore bearer	Type A
56 B	Pickled ripe olives	L, California	"Springer"	Abnormal	Soft and turbid brine	Negative	Spore bearing and gram-negative cocci	B. botulinus in enrichment also terminal end spore bearer	Type A
81 B	Pickled ripe olives	O, California	"Swell"	Bad	Soft	Negative	Rods and cocci	B. botulinus in enrichment B. sporogenes and terminal end spore bearer	Type B
105 a	Home canned asparagus	Washington	Bulging lid	Foul	Disintegrated stalk, turbid liquor	Positive M L D 1:50,000	Thermophiles and gram-positive rods	Pure B. botulinus	Type A
105 b	Home canned asparagus	Washington	Leaking	Foul	Disintegrated stalk, turbid liquor	Positive M L D 1:10,000	Negative	Pure B. botulinus	Type A
118	Commercially packed asparagus, 2 cans	California	"Swell"	Sour	Soft stalk	Negative	Thermophiles	Enrichmen pure B. botulinus	Type A
120 II	Commercially packed spinach, seized	Washington	Hard swell	Cheesy	Soft	Positive M L D 1:10,000	Sterile	Pure B. botulinus direct and enriched	Type A
120 III	Commercially packed spinach, seized	California	Hard swell	Cheesy	mushy	Positive	Sterile	Pure B. botulinus direct and enriched	Type A
114 C	Home canned fried chicken	California	Flat lid but leaking	Repulsive	Soft, but no liquor	Negative	Cocci and Molds	Enrichment B. botulinus and B. sporogenes	Type A
220	Home canned string beans, 2 cans	Oregon	Flat	Normal	Firm	Negative	Spore-bearing rods	Direct terminal and spore bearer, enriched pure B. botulinus	Type A
221	Home canned corn	Idaho	Bulging lid, leaking	Sour	Firm, dry, no liquor	Extract positive	Fine gram-positive rods	Direct pure B. botulinus	Type A
222	Five unprocessed "bulk olives"	California	....	....	Gas, turbid brine sediment, soft olives	Brine nontoxic	Variety of rods, cocci, etc.	B. botulinus, B. sporogenes, terminal end spores	Type A

mixed with beef heart peptic digest liver broth, exhausted and incubated as previously described. Tests for toxin and isolation of B. botulinus in pure culture are made according to the methods developed in this laboratory.

Bengtson[38] has suggested the intraperitoneal inoculation of white mice with the citrated blood of persons suffering from botulism in order to establish a differential diagnosis or to determine the type of intoxication. Kob,[40] in 1905, reported that the serum from a child collected on the 9th day after eating poisonous meat caused typical symptoms when injected into white mice intraperitoneally, and recently Semerau and Noack,[41] in Strassburg, claimed to have demonstrated by the inoculation of guinea-pigs the presence of toxin in the serum of 4 patients on the 4th, 6th, 9th, 16th and even on the 25th day after the consumption of the poisonous food. In America, this procedure has been tried on only one case (Report 80) with a negative outcome. These tests are of considerable clinical and scientific importance and should by all means be repeated when the occasion arises.

TABLE 19

B. botulinus in Human Necropsy Specimens

Outbreaks	Location	Specimens Examined	Toxin Tests with Salt Extracts	Positive Cultures	Type of B. botulinus
Report 7	Richmond, Calif.	Spleen, liver, lungs, kidneys, colon and upper ileum	Negative	Negative	
Report 15	San Rafael, Calif.	Spleen, liver and colon	Negative	Negative	
Report 68	Florence, Ariz.	Spleen, jejunal content, jejunal wall, brain	Negative	Intestinal wall	B
Report 98	Healdsburg, Calif.	Spleen, liver, transverse colon	Negative	Liver, transverse colon content and wall	A

EXAMINATION OF NECROPSY MATERIAL

In connection with medicolegal questions, systematic necropsy cultures should be carried out by means of dependable procedures. Revived interest in the relation of B. botulinus to certain animal diseases necessitates likewise the application of definite bacteriologic methods. We shall describe the procedures employed in the study of (a) human, (b) chicken and (c) horse and cattle necropsies.

(*a*) *Human Necropsies.*—Specimens from 4 necropsies have been examined. The pieces of spleen and liver were thoroughly sterilized by immersion in hot paraffin oil (180 C.). Samples varying from 25 to 150 gm. were ground with sand and thoroughly emulsified in salt solution. Intestinal chyme or dry fecal pellets adherent to the mucosa of the small and large intestines and portions of the intestinal mucosa were emulsified in salt solution. The emulsions were, in 2 instances, tested for toxins of B. botulinus and then heated for one hour at 70 C. and mixed with beef heart peptic digest liver broth. The enrichment cultures were tested after an incubation period of 10 days at 37 C. B. botulinus was readily isolated from these cultures. The results of these studies are shown in table 19.

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[40] Med. Klin., 1905, 1, p. 84.

[41] Ztschr. f. klin. Med., 1919, 88, p. 304.

Attention is called to the absence of B. botulinus in 4 spleen cultures. These observations are quite in harmony with the findings on experimental animals. Guinea-pigs which succumb to botulism following the ingestion of large amounts of detoxified spores of B. botulinus may furnish sterile splenic cultures, while the mesenteric lymphnodes, the liver and bonemarrow give positive enrichments. The demonstration of B. botulinus in the stools of patients with clinical cases suggests an examination of the colon and the rectum. The results obtained in a recent outbreak encourages the belief that a quantitative study of such specimens may furnish valuable information as to the possible growth of B. botulinus in the intestinal tube. From the standpoint of early diagnosis, it is possible that the presence of the ingested toxin might be demonstrated in vomitus or contents of the stomach by the inoculation of laboratory animals with filtrates.

TABLE 20

B. BOTULINUS AND ITS TOXIN IN CHICKEN NECROPSY SPECIMENS

Report*	Location	Specimens Examined	Toxin Tests with Extracts	Positive Cultures	Type of B. botulinus
16	Saratoga, Calif.	Spleen, liver, crop, gizzard, kidneys, lungs, intestines, 6th day of disease	Negative	Caeca, but not small intestine or colon	A
19	Los Angeles, Calif.	Spleen, liver, gizzard, crop, small and large intestines	Crop content negative	Spleen, liver, crop, gizzard, small and large intestines	A
26	Klamath Falls, Ore.	Spleen, liver, gizzard, crop, intestines	Crop content positive, but not intestines	Crop content only	A
51	Tieton, Wash.	Crop, gizzard, spleen	Crop content positive	Crop content only	B
53	Menlo Park, Calif.	Crops of 3 decomposed chickens, buried 5 days	Negative	Crops of all 3	A
92	San Jacinto, Calif.	Crops, gizzards, intestinal content, spleens and livers of 2 chickens	Crop of 1 chicken	Crops and contents of two, gizzard and intestines of one	A
105	Yakima, Wash.	Crop only	Positive	Crop	A
106	Glendale, Calif.	Crop, intestines and gizzard of one and crop content and wall of another	Negative	Crop content and wall of both	A
107	Fort Collins, Colo.	Crop content	Positive 0.01 c c for mouse	Crop and jar of corn	A

(b) *Chicken Necropsies.*—The importance of bacteriologic studies of cadavers of fowls has been discussed in previous papers. In a number of recent studies an attempt has been made to test the crop and intestinal content for the toxin of B. botulinus and to make cultures of the organs by means of enrichments. The entire content of the crop or gizzard is suspended in salt solution, thoroughly shaken and allowed to stand at room temperature for from 1 to 2 hours. The extract is filtered through paper and is either centrifugalized or passed through a Mandler candle. Mice or guinea-pigs are injected with or without antitoxin. Cultures of the organs are prepared according to the procedures mentioned under (a). The results obtained by those methods are presented in table 20.

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* The number refers to the reports mentioned in the paper: The Epidemiology of Botulism, to be published in a bulletin of the U. S. Public Health Service.

The demonstration of the toxin of B. botulinus in the crop has been possible in the cadavers of birds which have succumbed to the intoxication in from 24 to 48 hours. It is self-evident that the necropsies have been performed, as a rule, shortly after death. The excised tissues have been shipped separately in sterile containers. Toxin tests alone support the clinical diagnosis of botulism, while the isolation of B. botulinus from the crop or intestinal content lacks etiologic significance on account of the wide distribution of this organism in soil and its products with which chickens have intimate contact.

(c) Necropsy Specimens of Horses and Cattle.—In making cultures of organs or of the intestinal content of large domestic animals, painstaking attention has been paid to a thorough sterilization of the outside portion of the samples in order to avoid accidental contaminations. The tissues of 2 horses and 1 mule which succumbed to forage poisoning and of 2 cows which suffered from icterohemoglobinuria have been tested for toxin and cultures made by the methods described under (a) and (b). B. botulinus has been isolated from the intestinal content of the horses and the liver, mesenteric lymphnodes, etc., of 2 cows. The procedures have, therefore, proved satisfactory but have shown that B. botulinus is not infrequently a common inhabitant of the intestinal tract of domestic animals. In another paper it has been pointed out that an etiologic relationship of these organisms to the diseases or lesions from which they have been isolated is not proved. In future studies, therefore, an attempt should be made to demonstrate the toxin in the suspected feed or in the gastric content of the animals which exhibit symptoms of botulism.

SUMMARY

This paper describes the methods employed for the enrichment and demonstration of B. botulinus in specimens of soil and its products. The composition of the medium, the preparation and heating of the samples, the period of incubation and storage, the identification of the toxin and the isolation of the organism from toxic enrichment cultures are discussed and their value considered in the light of numerous experiments. It is emphasized that inexperienced workers should question their results until they eliminate the danger of laboratory contamination by continuous, painstaking vigilance, proper sterilization of the culture mediums, glassware, etc., and by repeated control examination.

The methods applicable for the examinations of suspected food, clinical and necropsy material are described in detail.

THE DISTRIBUTION OF THE SPORES OF B. BOTULINUS IN CALIFORNIA. II *

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In the course of an investigation of certain phases of botulism, it was realized that the prevention of this intoxication should not rest with an attempt to destroy the spores, but should go further and determine, if possible, the natural habitat of this formidable species. Such an inquiry, conducted in California, was not only imperative on account of the rather frequent occurrence of human and animal botulismus following the consumption of vegetable products grown and packed in this state, but appeared most promising on account of the preliminary studies conducted by G. S. Burke.[1] This worker found the spores of B. botulinus on apparently sound fruits, vegetables and feeds collected at widely separated localities in California. The organism was not in any way associated with active decay, although on one occasion a strain was isolated from the feces of a hog which had ingested spoiled, toxic peas four months previous to the collection of the manure specimen. This latter observation coincided with the report of Kempner,[2] who recovered, in 1897, a strain of B. botulinus from the intestinal content of a "normal hog" and indicated that at least in a small percentage of instances the spores, and perhaps the vegetative stages of this anaerobe may persist as saprophytes in the intestinal canal of animals.

The successful demonstration of B. botulinus on fruits, vegetables, etc., by Burke immediately suggested the existence of this anaerobe in the soil. From the facts available, however, it was by no means clear whether B. botulinus occurred only in cultivated regions or whether the bacillus was a part of the common soil flora and multiplied under natural conditions in forest and mountain soil. The failures of van Ermengem [3] to isolate B. botulinus from various earth samples, such as garden soil, dirt from streets, canal slime, mud from ponds and rivers, etc., together with the relatively rare occurrence of botulismus

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Received for publication, July 26, 1922.

* This study was aided by grants from the National Canners Association, the Canners League of California and the California Olive Association.

[1] Jour. Bacteriol., 1919, 4, p. 541.

[2] Ztschr. f. Hyg. u. Infektionkr. 1897, 26, p. 482.

[3] Handbuch d. pathog. Mikroorganismen, 1912, 4, p. 936.

in certain states of the Union, indicated that the anaerobe was probably not omnipresent but occurred only in certain soils, probably due to accidental contaminations from heavily infected areas or possibly manure or animal excreta. These and similar considerations prompted an extensive cultural study of the telluric distribution of B. botulinus. Without an adequate knowledge of the biochemical activities and growth requirements of the bacillus at the time these studies were begun, it was naturally impossible to foresee the scope of the investigation. When the problem was approached, the prevailing idea of the intestinal habitat of sporulating anaerobes prompted the examination of cultivated and manured garden, field and pasture soils. Considerable attention was paid to the distribution of B. botulinus in various olive groves and on vegetables and fruits bought in the open market of various cities and towns of the state. The examination of control specimens and with it the frequent demonstration of B. botulinus in uncontaminated mountain soil, however, changed the original point of view so that, in addition to sampling field specimens in the vicinity of rural communities in which human or animal botulism had occurred, soil from uninhabitated, virgin territories was collected and cultures made. Simultaneously, numerous specimens of manure were studied, and the persistence of the spores of B. botulinus in the intestines of laboratory animals, as well as the factors responsible for the growth of the anaerobe in various earth specimens under laboratory conditions, was experimentally investigated. In order to verify some of the observations made in California, a broad investigation of soil and vegetable samples procured from every state of the Union, Canada, Hawaii, Alaska, China and Europe was subsequently made. This paper deals with the findings made on 607 specimens of soil and vegetables collected in California, and is mainly intended to serve as an introduction to the communications dealing with the distribution of the spores of B. botulinus in the United States.

Collection and Study of Samples

Soil specimens (200-500 gm. samples) were collected in small, carefully sterilized, sanitary tin containers (No. 1 cans). The lids of the containers were sealed and covered by sterile linen cloth. As a rule, samples of surface and subsurface soils (about 10 inches) were procured. Vegetables and fruits, as found in the open market or gardens, were packed in tight wooden boxes. Extreme precautions were taken to avoid extraneous contaminations in transit, as well as in the laboratory.

The writers are deeply indebted to Dr. J. C. Geiger, Epidemiologist of the U. S. Public Health Service, who carefully collected the majority of specimens on which these studies are based. A few samples were obtained by the Inspectors of the Food and Drug Department, California State Board of Health, by collaborators, by assistants and by veterinarians, who reported cases of "forage poisoning."

Adequate samples, varying from 10 to 100 gm. of soil, vegetables, etc., were treated according to the procedures outlined in a former paper.[4] All cultures were made with heated specimens in beef heart peptic digest liver broth. A number of the early cultures were incubated for from 4 to 6 weeks instead of 10 days, this shorter period was later adopted as a standard procedure. It is, therefore, not unlikely that on account of the deterioration of the toxin following the longer periods of incubation some of the specimens containing the spores of B. botulinus were not recognized. The cultures were always tested on guinea-pigs and the type of toxin determined on the same species of animal. About one-third of the toxic cultures produced characteristic symptoms of intoxication, but the nature of the toxin could not be ascertained by an antitoxin neutralization test. Sufficient experimental evidence has, however, been collected and presented in the first paper of this series, to support the conclusions that those soil or vegetable samples producing weakly toxic cultures contained only a few spores of B. botulinus. In a study dealing with the distribution of B. botulinus in nature, the findings pertaining to these weakly toxic specimens, even if they are not conclusively proved to be the result of B. botulinus by antitoxin tests, deserve recognition and are therefore included in the discussion.

EXPERIMENTAL DATA

The survey made in California during the years 1920 to 1922 covered the following phases of the problem: (1) A search for the spores in the soil or its products collected from gardens in widely separated localities in which human or animal botulism had occurred during the years 1919 to 1922; (2) systematic sampling of soil, vegetables and fruits from olive groves, spinach fields, which had furnished the raw materials, and later of products of packing plants, such as improperly sterilized canned products which had caused several outbreaks of human botulism; (3) culturing of fruits and vegetables bought in the open market; (4) toxicologic and cultural tests of feeds, hay, etc., supposed to have caused forage poisoning; (5) culturing of manure used in the fertilization of olive groves, vegetable gardens, etc.; (6) selective sampling and making of cultures of virgin soil collected in isolated areas of the Sierra Nevada, San Bernardino and Coast Range Mountains.

It is unnecessary to describe the various specimens and the time of the year during which the material was collected and cultures made. The results are summarized in table 1. For future reference, a presentation along geographic lines has been chosen and the findings previously reported by Dickson and Burke are included in the table. The survey is obviously not exhaustive, but the ubiquitous recognition of the spores in every section of the state made it unnecessary to extend the examination along broader telluric lines, although some of the early studies suggested a localization of the spores in certain districts and counties. This belief that the distribution of B. botulinus is confined to certain areas, towns, etc., followed the examination of a limited number of specimens and was readily disproved by the subsequent broader study of the problem. From a biologic point of view, intensive sampling of

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[4] Jour. Infect. Dis., 1922, 31, p. 501.

TABLE 1

RESULTS OF TESTS WITH SPECIMENS FROM VARIOUS COUNTIES IN CALIFORNIA

No.	Record	Location	Character of Specimen	Number of Samples	Number of Cultures	Total Number of Typed Cultures	Results				Remarks
							Untyped	Type A	Type B	Types A and B	
		Alameda County									
1	182C	Grizzly Peak, Berkeley Hills (1,759 ft.)	Virgin soil from new trail...	6	6	5	—	5	—	—	
2	33B	Oakland..............	Hay (2) and chicken manure	3	3	—	—	—	—	—	
3	15	Berkeley..............	One jar of spoiled string beans	1	2	2	—	2	—	—	
*	Report	Berkeley..............	Three jars of string beans and gizzards of 2 chickens	5	5	5	—	—	5	—	Isolated by E. C. Dickson
		Butte County									
4	4	Olive ranches, Oroville	Olives on trees, bird pecked and buried, hog manure	6	12	5	—	—	5	—	
5	81	Packing plant, Oroville	Decayed Sevillano and dried Mission olives	3	3	—	1 (Sevillano)	—	—	—	
6	81B	Packing plant, Oroville	Swelled cans of ripe pickled olives	3	3	1	—	—	1	—	Brine nontoxic
7	34	S. Ranch, Oroville.....	Soil (5), green olives and leaves (1)	6	15	—		—	—		Incubated for 12 weeks
8	35	O. Ranch, Oroville.....	Soil (6), green olives and leaves (1)	7	12	—	2 (soil)	—	—	—	Incubated for 12 weeks
9	36	P. Ranch, Oroville.....	Soil (7), green olives and leaves (2)	9	20	—	—	—	—	—	Incubated for 12 weeks
10	38	D. Ranch, Oroville.....	Soil (6), green olives and leaves (2)	8	18	—	—	—	—	—	Incubated for 12 weeks
11	39	E. Ranch, Oroville..... (1920)	Soil (6), manure (1), olives and leaves (2), fertilizer (1)	10	14	—	1 (soil)	—	—	—	One fertilizer positive; manure malignant edema
12	184	E. Ranch, Oroville..... (1921)	Soil, fertilized with manure	6	6	2	—	2	—	—	
13	52-54 IV	Chico..................	Hay suspected of causing forage poisoning	4	4	—	—	—	—	—	Extract nontoxic
14	68B	Chico..................	Hog manure...............	—	—	—	1	—	—	—	
15	62B	Chico..................	Mill run and hog tankage...	2	4	—	1	—	—	—	
16	67	Chico..................	Soil......................	16	16	—	4	—	—	—	Incubated for 28 days
17	68	Biggs..................	Soil and decayed chickens (4) poisoned by spoiled corn	5	8	(chicken yard)	1 (crop) 1 (corn garden and burial ground)	2	—	—	
18	73	Durham................	Hay, alfalfa, maize, calf-meal, rice	8	8	—	—	—	—	—	
19	100B	Packing plant, Oroville	Spoiled, pickled olives.......	9	9	—	1	—	—	—	

		Colusa County									
20	10	Colusa	Decomposed hay (2), moldy beanstalk and plant (2), soil (2)	6	10	1 (bean plant)	2 (soil)	1	—	—	
		Contra Costa County									
21	20	Pleasant Hill	Decomposed hay (forage poisoning)	—	—	—	—	—	—	—	Malignant edema
		El Dorado County									
22	171	Lake Tahoe	Virgin soil	5	5	5	—	5	—	—	
		Fresno County									
23	76	King's River Canyon	Spleen, intestine, liver, etc., of a horse	6	6	1 (intestines)	—	1		—	Forage poisoning (?)
24	76B	King's River Canyon	Soil from pasture, hill top	3	3	3	1	3	—	—	
25	76A	Fresno hills	Soil	3	3	—	—	—	—	—	
26	76B	Mountain valley	Virgin soil	3	3	1	—	1	—	—	
27	180A	Telden Pass	Virgin-soil	1	1	—	—	—	—	—	
		Glenn County									
28	180	Orland	Hay and feed	2	2	—	—	—	—	—	
		Humboldt County									
29	77	Eureka	Soil from vegetable gardens	8	8	—	—	—	—	—	Incubated for 28 days
30	77B	Eureka	Vegetables from open market	10	10		2 (parsnips)	—	—	—	
31	77B II	Eureka	Soil from vegetables	2	2	—	—	—	—	—	
		Inyo County									
32	180A	Piute Pass	Virgin soil	1	1	—	—	—	—	—	5 gm. sample
		Kern County									
33	66B	Bakersfield	Straw and burned hay (forage poisoning)	2	4	—	—	—	—	—	
		Los Angeles County									
34	5	Long Beach, La Mosa Ranch	Moldy olives (3), soil (3)	6	6	—	—	—	—	—	
35	24A	52d St., Los Angeles	Garden soil, upper and lower layers	6	6	—	—	—	—	—	
36	26	Whittier	Hay (1), soil (1)	2	4	—	—	—	—	—	
37	29A	Whittier	Manure of sick horses	2	2	—	—	—	—	—	
38	54	Whittier	Hay (2), manure (2), corn (3)	7	7	4 (hay and manure)	—	2 (manure)	2 (hay)	—	
39	74A	Whittier	Hay (1), manure (2), barley (1)	4	4		1 (hay)	—	—	—	
40	50	Los Angeles	Cadaver of chicken	1	11	1 (9)	—	1 (9)	—	—	
41	50	Los Angeles	Chicken feed	2	2		—				
42	50	37th Ave., Los Angeles	Soil and string beans	2	2		—	—			See Human Reports 18 and 19
43	58	Sylmar Ranch	Soil	3	6	1	1	1	—	—	
44	58	Sylmar Ranch	Manure (2), Mission olives from trees	5	10		3 (olives, tree and manure)	—	—	—	
45	58	Sylmar Ranch	Soil	5	10	2	4	2	—	—	

TABLE 1—*Continued*

Results of Tests with Specimens from Various Counties in California

No.	Record	Location	Character of Specimen	Number of Samples	Number of Cultures	Total Number of Typed Cultures	Results				Remarks
							Untyped	Type A	Type B	Types A and B	
		Los Angeles Co.—Cont.									
46	58	Sylmar Ranch	Olives from trees, leaves, etc.	4	8	1	2	—	1	—	
47	129B	51st St., Los Angeles	Surface soil, chicken droppings, goat manure	4	4	—	2 (soil)	—	—	—	See Chicken Outbreak 21
48	129	Laguna Ranch, Los Angeles	Feed, manure, stagnant water, sewage (5)	9	9	—	4 (manure and beet pulp)	—	—	—	Bovine botulism suspected
49	134	New Hall Ranch	Soil (6), manure (2), decayed meat (1)	9	9	—	—	—	—	—	Malignant edema
50	135	New Hall Ranch	Pasture soil	12	12	1	4	—	1	—	Five vibrion septique
51	139	Los Angeles	Barn feed and manure	2	2	—	—	—	—	—	
52	181	Los Angeles	Corn husks and potatoes	3	5	1	—	1	—	—	
53	224	Glendale	Three chickens and home canned corn	4	1 (6)	1 (6)	—	1 (6)	—		See Chicken Outbreak 107; isolated
54	136	Pasadena	Sewer farms, soil and sewage	4	4	—	2 (soil)	—	—	—	
55	196	Mount Lowe (6,100 ft.)	Soil (5), trail dust (1)	6	6	2	1	2	—	—	Trail dust contaminated with horse feces contained B. tetani
		Marin County									
56	7	San Rafael	Beanstalks (1), soil (2)	3	5		—	—	—	—	See Human Report 15
57	8	San Rafael	Beanstalks and soil	2	4	—	—	—	—	—	
58	25	San Rafael	Soil	5	10	—	—	—	—	—	
59	K2	San Rafael	Specimens of vegetables and fruits (cherries, beans, been root, asparagus, carrots)	12	25	3	3 (beans, vine, asparagus)	2 (asparagus)	1 (asparagus)	—	
60	181A	San Rafael	Two fresh clams	2	2	2	—	2	—	—	
61	202	Mt. Tamalpais	Virgin soil from Dipsea trail, Lone Pine, Forest Deer Park and Alpine trail	8	8	5	—	2 Lone Pine and Alpine trail 1 Forest Deer Park	—	2 Forest Deer Park and Dipsea	
		Mariposa County									
62	52B	• Yosemite Valley	Virgin soil, Camp C.6, El Capitan Meadow and Ranger Station on Bridal Veil	4	8	2	—	2 (Camp 6)	—	—	
63	122	Yosemite Valley	Rockslide virgin soil, 1,000 feet above valley	4	8	3	1	3	—	—	
64	122	Yosemite Valley	Excavation in virgin soil 1 foot deep	6	6	6	—	6	—	—	
65	171	Gentry	Virgin soil	8	8	2	—	1	1	—	

		Mono County								
66	171	Mono Lake	Virgin soil	6	6	2	1	2	1	—
		Napa County								
67	42	Napa	Garden soil and vegetables	13	13		1 (bean leaves)	-	—	—
68	47	Calistoga	Garden soil, vegetables, hog and horse manure	10	10	—	—	—	—	—
69	114	Napa	Soil from horse corral	2	2	—	—	—	—	—
		Placer County								
70	79B	Colfax	Garden soil (2), pasture soil (2)	4	4	—	—	—	—	—	Malignant edema; garden soil
71	172B	Roseville	Corral dirt and water (forage poisoning)	4	4	—	—	—	—	—
		Plumas County								
72	183	Feather River Canyon	Virgin soil	12	12	7	—	6	—	1
		Riverside County								
73	135	Riverside	Soil from orange groves, garden 1, Mt. Rubidoux	5	5	—	—	—	—	—
		Sacramento County								
*	Report	Sacramento	Jar of string beans, locally grown	—	—	—	—	—	1	—	1918, Mrs. G. S. Burke
		San Benito County								
*	Report	Hollister	Water flies, slugs, sowbugs, earthworms, chicken manure, straw, dirt, mud, hog manure	—	—	1	—	—	1 (hog manure)	—	1918, Mrs. G. S. Burke
		San Bernardino County								
74	135B	Cucumbo	Soil from gopher holes	2	2	—	—	—	—	—
75	136	Sewer farms	Soil, top and deep	6	6	2	2	—	2	—	Spinach responsible for outbreak 63 grown in these farms
		San Diego County								
76	55	San Diego	Soil, weeds and goat manure	6	8	4	—	4 (top soil and weeds)	—	—	Suspected botulism among goats
77	58	Escondido	Soil (3), potatoes (1), beans (1), hog manure (1)	6	13	2	—	2 (bean garden soil and hog manure)	—	—
		San Francisco County								
78	28	Golden Gate Park	Fertilized soil, deep and surface	5	10	—	—	—	—	—
79	32	Golden Gate Park	Fertilized soil from nursery	5	10	—	—	—	—	—
		San Joaquin County								
80	62C	Stockton	Damp hay	1	3	—	—	—	—	—
		San Mateo County								
81	46	Menlo Park	Cadavers of chickens and soil	5	10	1	—	1	—	-	See Chicken Report 53
82	49	Menlo Park	Soil (8), bean leaves (1)	9	9	—	—	—		-	Incubated for 10 weeks

TABLE 1—*Continued*

RESULTS OF TESTS WITH SPECIMENS FROM VARIOUS COUNTIES IN CALIFORNIA

No.	Record	Location	Character of Specimen	Number of Samples	Number of Cultures	Total Number of Typed Cultures	Results				Remarks
							Untyped	Type A	Type B	Types A and B	
		Santa Barbara County									
83	9	Santa Maria..........	Straw (forage poisoning)...	1	2	1	—	—	1	—	
84	24	Santa Maria..........	Hay (1), soil (3)............	4	4	3	—	—	3 (soil and hay)	—	
85	127	Montecito.............	Peas, foliage and soil (2)...	4	4	3	—	2 (soil)	1 (foliage)	—	
86	127B	Santa Barbara........	Virgin soil and olives (San Carlo Ranch)	3	5	5	—	5 (olives, leaves and soil)	—	—	
87	202B	Montecito.............	Soil from Bishop Ranch....	1	1	1	—	—	—	1	
		Santa Clara County									
88	1	Saratoga.............	Chicken cadaver...........	1	10	1	—	1 (intestines)	—	—	
89	2	Saratoga.............	Garden soil, string beans...	2	12	2	—	2 (soil)	—	—	
90	40	Saratoga.............	Soil (7), fruits (4), vegetables (6), leaves (2), sewage	20	27	1	—	1 (soil, orchard)	—	—	
91	31	Morgan Hill..........	Fruits and vegetables.......	5	8	1	1	1 (apricot)	—	—	
92	43	Los Gatos..........	Soil (4), plums (2), bean leaves	7	9	—	4 (soil)	—	—	—	
93	61	San Jose..............	Vegetables from open market	15	15	5	2	5 (beans, celery, potato, turnips, lima beans)	—	—	
*	Report	San Jose..............	Chicken cadaver...........	—	—	—	—	1	—	—	Isolated by E. D. Dickson, see Report 1
*	Report	Palo Alto...........	Peas, beans, peaches, caterpillar, corn, cherries	—	27	2	—	2 (cherries)	—	—	Isolated by Mrs G. S. Burke
		Santa Cruz County									
94	37	Boulder Creek.........	Garden soil and bean leaves	5	5	—	—	—	—	—	
		Shasta County									
95	70B	Redding...............	Virgin soil..................	6	6	—	1	—	—	—	
		Sonoma County									
96	219	Sebastopol Healdsburg	Spoiled string beans, locally grown	1	1	1	—	1	—	—	
		Stanislaus County									
97	113B	Newman...............	Garden soil.................	6	6	6	—	6	—	—	
*	Report	Oakdale...............	Spotted leaves, pods, bush string beans, ants, spiders, bugs, grass hoppers	—	23	1	1 (spider, etc.)	—	1 (spotted bean leaf)	—	1918, Mrs. G. S. Burke
*	Report	Oakdale....	Moldy hay..................	1	1	1	—	1	—	—	1918, Mrs. G. S. Burke

		Tehama County									
98	64	D. Ranch near Corning	Soil (4), bird pecked ripe olives	6	13	–	–	—	—	—	
		Tulare County									
99	51	Lindsay	Sh. Ranch soil (7), green olives (5), leaves (1)	13	13	1	1	—	1 (soil)	—	
100	51	Lindsay	G. Ranch soil (2), green olives (4)	4	4	1	—	1 (soil)	—	—	
101	51	Lindsay	Dr. Bruce soil (2), green olives (4)	6	6	—	—	—	—	—	
102	51	Lindsay	I. Ranch, oives and leaves	2	2	2	—	—	2 (leaves and green olives)	—	
103	51	Lindsay	Eck. Ranch soil (2), olives (2)	4	4	—	2 (soil and olive)	—	—	—	
104	51	Lindsay	Eis. Ranch soil (1), olives (2), manure (2)	5	5	—	—	—	—	—	
105	51	Lindsay	Orange grove soil	2	2	—	—	—	—	—	
106	51	Packing plant, Lindsay	Scrapings pickling barrels (2), shipping barrels (1), olive dumps (1), conveyors (2), discarded olives (7)	13	13	3	1 (discarded olives)	1 (scrapings shipping barrel)	—	—	
		Tuolumne County									
107	171	Tioga Pass	Virgin soil	6	6	2	—	2	—	—	
		Ventura County									
108	41	Oxnard	Soil (1), hay (1), horse manure (1)	3	6		—	—	—		
		Yolo County									
109	80	Davis	Horse (2) and hog (2) feed, manure (4)	8	22	—	—	—	—	—	
110	87	Davis	Horse, hog and cow feed, manure	8	8	—	—	—	—	—	
		Unassigned—General Distribution									
111	22	San Francisco	Vegetables (2), fruits (1)	4	10	2	—	—	—	—	Toxin deteriorated befor typing
112	1K	San Francisco	Fruits (11)	11	16	3	3 (turnip tops, plums)	2 (turnips, cherries)	1 (peaches)		
113	27	San Francisco	Vegetables	6	14	2	2 (radishes, peas)	—	2 (beets)	—	
114	30 and 33	San Francisco	Spinach, string beans, etc.	11	11		—	—	—	—	Incubated for 6 weeks
115	49B	San Francisco	Beet tops, corn, potatoes	4	6	2	1 (corn)	—	2 (beet tops)	—	

certain mountain areas deserves attention in the near future, but from a practical standpoint, at least, it is unnecessary to devote material and energy to an additional study of specimens collected in California. It has been definitely proved that the spores are widely distributed and may be found in soils and their products from any district or locality.

An analysis of the data presented in table 1 shows that 624 specimens have been examined by means of 894 cultures. These figures include also the samples of spoiled home canned products, tissues, etc., procured from animals after necropsies had been performed. Six hundred and seven specimens, representing samples of soil, vegetables, feeds, fruits and manure, were selected for further consideration and discussion; 179, or 29.4% of the specimens produced toxic cultures; 89 contained type A; 26 type B, and 4 type A and B B. botulinus toxins, while 60, or 9.8% of the cultures were not sufficiently toxic to furnish conclusive antitoxin neutralization tests. One hundred and nineteen, or 67.6% of the 179 toxic cultures were properly identified. Nearly three-fourths, or 74.8% of the typed cultures contained type A, showing clearly the predominance of this resistant type in the soil of California. Type B was found in 22.1% and a mixture of types A and B in 3.1% of the samples. The latter findings are important, as they definitely indicate that the 2 types of B. botulinus may coexist in the soil. The real biologic significance of the tabulated data was, however, not realized until the cultural findings were classified according to their origin. This classification is presented in chart 1.

For the sake of clearness, it is considered advisable to discuss the various columns separately.

1. *Virgin Soil Specimens.*—Only samples of soil collected from areas definitely known to be free from contaminations of animal excreta are included in this group; in fact, the majority of specimens represent earth of the Sierra Nevada or Coast Range Mountains. The first positive proof that B. botulinus exists in virgin soil was furnished by a number of soil specimens removed from a rockslide which occurred March 1, 1921, about 1,000 feet above the floor of the Yosemite Valley (El Capitan side, Big Oak flat). Samples were taken on April 20. Repeated cultural tests revealed the existence of spores of B. botulinus; the organism being isolated in pure culture on numerous occasions. Identical findings were made with soil collected from an excavation 4 feet deep in the heart of the valley, in which pollution with manure, etc., was absolutely excluded. These findings have subsequently been strengthened by the examination of soil specimens from the Sierra Nevada ridge near Tioga Pass, the Mono Lake and Lake Tahoe region, Feather River Canyon, etc. A total of 78, with 45, or 57.6%, toxic cultures has been studied. Forty-three samples of the latter cultures have been identified, and the predominance of type A has been definitely established. One sample, which originated from the vicinity of Gentry, Sierra foothills, Yosemite National Park, contained B. botulinus type B, while 2 specimens from Mount Tamalpais, near San Francisco, and 1 from the mouth of the Feather River Canyon contained mixtures of types A and B. Moreover, it is evident from the ease with which B. botulinus was isolated from the soil enrichment cultures and from the relatively low percentage of weakly toxic cultures that the spores are quite numerous in the virgin soil specimens. The evidence strongly supports the contention that B. botulinus is a common member of the soil flora in the Sierra Nevada and Coast Range Mountains.

2. *Garden, Orchard and Cultivated Field Soil Specimens.*—The samples included in this column originated from soil areas which were known to have

been fertilized either quite recently, or at least in the course of the last two to three years. In some instances, commercial fertilizer, but usually barnyard manure, had been employed. The majority of specimens were collected in olive groves and vegetable gardens. Recently a number of farms growing spinach and regularly flooded with sewage from Pasadena and other places have been tested. Fifty-nine cultures, or 26.9%, of the total 226 specimens were toxic. A relatively high percentage (40.6%) of the cultures was weakly toxic and could not be identified by antitoxin tests. These results are due to one or two conditions: either the number of spores of B. botulinus per gram of soil are few, or the concomitant anaerobes outgrow the toxin producers in the enrich-

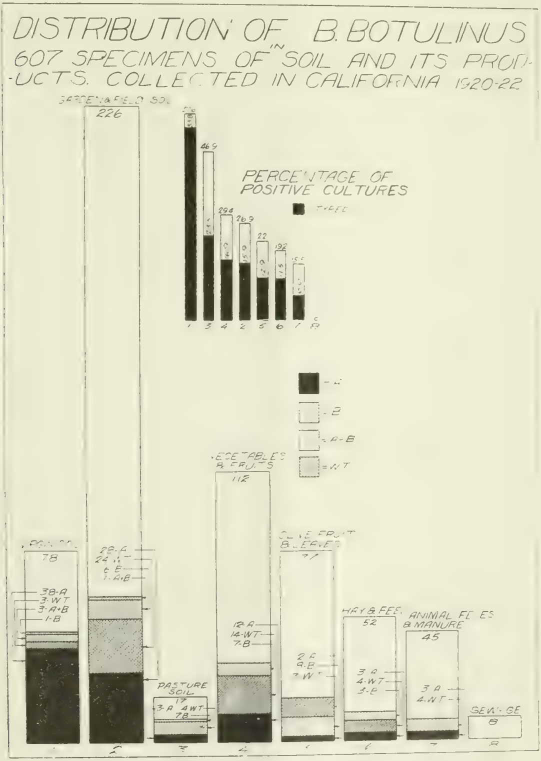

Chart 1

ment cultures. Type A was the predominant type, although type B appeared more frequently than in the virgin soil samples. The proportion of type A to type B for the virgin soil specimens was 38:1, while it shifted to 4.66:1 for the garden and cultivated field soil samples. The ranches from which the samples were taken are located in the Sacramento or San Joaquin Valley and receive their irrigation water from the Sierra Nevada Mountains. It is not unlikely that the fields are seeded with the type A spores from the water sources which are heavily infected with this organism. What happens to these spores can only be discussed after the various other columns have been carefully analyzed. It must, however, be concluded that the spores of B. botulinus are less frequently encountered in garden or cultivated field soil than in virgin mountain soil.

3. *Soil of Pastures.*—A small number of soils collected from pastures have been studied for the presence of B. botulinus. The specimens were collected from places on which horses or cattle had died from diseases diagnosed by local veterinarians as "forage poisoning." In every instance, the soil was markedly polluted with animal excreta, and it is significant that 9 of the 17 specimens produced "malignant edema" in guinea-pigs. Three of the 17 cultures contained type A, while one was neutralized by a type B antitoxin; 4 cultures could not be identified. Unfortunately, control tests of pasture soils not contaminated with animal manure were not made, and the limited number of specimens does not permit any definite conclusions.

4. *Vegetables and Fruits.*—This group of cultures was prepared with specimens either bought in the open market of San Francisco, San Jose (Santa Clara Valley), Los Angeles, Eureka (Humboldt County), or collected in gardens which had supplied the vegetables responsible as canned goods for cases of human or animal botulism. Cultures have been taken from potatoes, beets, asparagus, etc., heavily covered with soil, or fruits picked from the trees slightly contaminated with dust. In this connection it was noted that for example bird pecked cherries or bruised, partially spoiled apricots gave positive cultures more frequently than sound fruit; on one occasion, the soft, moldy blemish of an apricot gave a toxic culture, while the sound skin furnished a nontoxic culture. It is not unlikely that the spores of B. botulinus in the dust of the soil are fixed by the sticky sap of the bruised fruit. Thirty-three, or 29.4%, of the 122 specimens of vegetables and fruits produced toxic cultures. Fourteen, or 42.4%, of the cultures were weakly toxic and non-neutralizable, while 12 were neutralized by type A and 7 by type B antitoxins. The findings on the vegetables and fruits correspond in a general way with those made on the soil specimens collected in gardens and cultivated fields. The relatively high percentage of type B toxins has been repeatedly confirmed by the isolation of B. botulinus type B from the enrichment cultures. The vegetables, such as beets, asparagus, etc., bought in San Francisco were grown in truck gardens heavily fertilized with hog manure. Specimens of the fertilizer were tested for spores of B. botulinus with negative results. Soil products procured from plots recently planted with vegetables furnished enrichment cultures which contained type A toxins. Invariably the repeated examination of the soil demonstrated the same type. So far as the limited material permits of deductions, it is evident that the products of a soil which has been manured and cropped for many years more frequently contains the spores of B. botulinus type B than those of type A. Fruits and vegetables are excellently suited to demonstrate this distribution, probably on account of the relative minority of other proteolytic anaerobes which ordinarily destroy the toxins or overgrow B. botulinus present in specimens prepared from manured or fertilized soil.

5. *Olives, Foliage from Trees, Specimens from Olive Packing Plants.*—As a number of tragic human botulism outbreaks due to pickled ripe olives instigated the investigation on the distribution of B. botulinus in nature, it became necessary to follow the path of this organism from the olive grove to the packing plant. From the data summarized in column 5, it is evident that B. botulinus was readily demonstrated on olives. Not only were the leaves and fruits on the trees found to be contaminated, but the scrapings from the shipping barrels transporting the ripe olives from the groves to the cannery, as well as the refuse dumps composed of spoiled, discarded olives, supplied toxic cultures. Furthermore, several tins of ripe pickled olives, processed at low temperatures and unprocessed, so-called "bulk olives," gave pure cultures of B. botulinus type A. The frequent finding of type B spores only on the foliage and green fruits is closely connected with the predominance of this type in the manured soil of the ranches. This type is probably not very resistant against physical and chemical agents, rarely survives the pickling and preserving processes and has, therefore, not been found in the packed products.

Seventeen, or 22.0%, of the 77 olive specimens produced toxic cultures; the proportion of type A to type B was 1:4. The foliage and green olives collected from the trees furnished 10% weakly toxic enrichment cultures. From a practical standpoint, it is beyond question that B. botulinus is frequently carried on the ripe fruits into the shipping barrels, pickling vats and the packing plant. The question: Why did the organism under these prevailing conditions produce its toxin only in a very few nonsterile glass containers (probably 9 to 10 containers) is certainly not solved by the foregoing findings. It is not our purpose to enter into a theoretical discussion of a problem which was unfortunately not investigated when the conditions in the packing plants offered excellent opportunities for interesting disclosures. It is not unlikely that during and shortly after the war, the collection of ripe olives in the orchards was not conducted with a great deal of care. The raw product, heavily contaminated with soil, underwent further deterioration and the resistant spores were permitted to increase by prolonged storage in shipping barrels and pickling vats. The common practice of preserving the olives by heat not exceeding 212 F. produced in some containers an environment suitable for the growth of B. botulinus and its toxin. It is, however, now fully recognized that efficient sterilization of fresh and carefully handled ripe olives will prevent further outbreaks of botulismus.

6. *Hay, Straw and Animal Feeds.*—One-half of the specimens and cultures from which this column is computed originated from outbreaks of "forage poisoning," while the other half consisted of similar material collected in the vicinity of the outbreaks. Watery or saline extracts were usually found to be nontoxic for guinea-pigs, although 10, or 19.2%, of the 52 samples produced toxic cultures. Type A, as well as type B B. botulinus were demonstrated and isolated from the cultures. The percentage of positive cultures and the relative proportion of the two types of B. botulinus differed in no way from that established for the soil supplying the hay or feed. Furthermore, no differences were noted between the feeds suspected of having caused forage poisoning and those procured from sound haystacks or feeding troughs. The assumption that the finding of B. botulinus in the feed proves the botulismus nature of "forage poisoning" is not supported by the foregoing observations. In fact, it is shown that the mere cultural demonstration or isolation of B. botulinus in feeds, vegetables, etc., cannot be considered diagnostic of botulism.

7. *Manure and Fertilizers.*—Three, or 6.6%, of the 45 specimens contained the spores of B. botulinus type A. Four samples furnished weakly toxic enrich-

ment cultures. As a rule, large specimens varying from 100 to 500 gm. were employed. The low percentage of positive cultures is either due to an absence of the spores or the methods employed are unsuitable to detect the organism when present in relatively small numbers. It is not intended to discuss here the importance of some of the herbivorous animals as carriers or disseminators of B. botulinus spores, but the evidence strongly suggests that manure or fertilizers contribute relatively little to the pollution of the soil with this bacillus. On numerous occasions, parallel examinations of ranch soil and its fertilizing material revealed the presence of B. botulinus in the former and its absence in the latter. Moreover, it has been repeatedly pointed out that earth contaminated with animal feces is rarely infected, while mountain and forest soil in California are practically always infected with B. botulinus spores. In this respect the organism differs from its related species the tetanus bacillus. (See 54, Table 21.) It is not unlikely that occasionally the spores ingested with the feed are eliminated in the feces and in this manner herbivorous animals may act as distributors of the spores. Experimental studies to be presented by E. J. Easton in another paper confirm this conclusion and amplify the data outlined in this paragraph.

8. *Sewage.*—An extensive outbreak of botulism in chickens was investigated from both a pathologic and a bacteriologic standpoint. Vegetable and soil cultures revealed the fact that the garden in which the beans responsible for the fatalities were grown had been regularly fertilized by means of sewage and contained the spores of B. botulinus, type A. It was later found, however, that certain truck gardens furnishing spinach to canneries were constantly treated with sewage. Furthermore, a number of specimens of heated and unheated sewage were cultivated with entirely negative results, so that the evidence deduced from this outbreak among chickens can hardly be considered strikingly opposed to the previously established conception of the telluric origin of B. botulinus. It has been demonstrated in this laboratory that human excreta produce, as a rule, nontoxic enrichment cultures, irrespective of the fact that spores are frequently ingested on raw vegetables and fruits. Evidence is still lacking to prove that multiplication takes place in the human intestinal canal or in sewage and that the latter contributes to the contamination and pollution of the soil in certain districts or areas of this state.

In the light of the findings discussed under 7 and 8, it is evident that in California at least B. botulinus is not disseminated by animal excreta nor is the intestinal canal the natural habitat of this organism. This conclusion will be supported by data presented in subsequent papers, but it appears of interest to investigate briefly the distribution of the two types of B. botulinus as determined by this study. The predominance of type A in virgin soil and the occasional or relatively frequent occurrence of type B in manured gardens or ranch soil is sufficiently constant to suggest the existence of a biologic phenomenon. Data to be mentioned in a paper dealing with the morphologic and biochemical activities of 100 strains of B. botulinus indicate that the B types more often show variations in their cultural and serologic characteristics than the A types. In brief, it is believed that the former types are mutants of the latter. The biologic, physical and chemical influences encountered by the stable or fixed type A in cultivated and manured soil are probably responsible for the creation of a new adaptation type which can develop more readily in the new environment and as such preserve the genus Botulinus. The mutation is in this case favorable to the existence of the organism and enables it to multiply actively in an environment which is less suitable than the virgin, untilled soil. This interpretation of the origin of type B is quite in accordance with the observation that proteolytic anaerobes mutate more frequently than nonproteolytic types. One recalls in this connection the recently published paper of H. H. Heller[5] on mutations in the genus B. tetani and some of the studies of Löhnis[6] dealing with the life cycles of soil bacteria. It is obvious that this explanation deserves careful experimental investigation and is herewith offered as a working hypothesis for a field which has not only biologic but also some practical importance.

CONCLUSIONS

The examination of 624 specimens of soil, vegetables, fruits, feeds, manure and sewage collected in 36 counties of California and studied by means of 894 cultures definitely indicates that the spores of B. botulinus are very widely distributed. Approximately 30% of the samples produced toxic cultures. 74.8% of the identified toxins were neutralized by a type A, 22.1% by a type B and 3.1% by a polyvalent antitoxin.

The evidence strongly suggests that the natural habitat of B. botulinus is found in virgin mountain or forest soil. B. botulinus is also present in cultivated garden and field soils and their products. Vegetables and fruits bought in various cities and towns of California carry the spores of B. botulinus.

B. botulinus type B occurs predominantly in cultivated and manured soils and is probably a mutant of the fixed type A.

[5] Jour. Infect. Dis., 1922, 30, p. 33.

[6] Jour. Agric. Res., 1916, 6, p. 675.

THE DISTRIBUTION OF SPORES OF B. BOTULINUS IN THE SOIL OF A RESTRICTED AREA IN CALIFORNIA. III *

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Since the fatal outbreak of poisoning in 1919 in various parts of the United States due to the consumption of California olives which had been contaminated by B. botulinus, a widespread interest has been aroused, particularly in this state, as to the distribution of the spores of this bacillus. Aside from their desire to suppress this menace to the public health, various organizations, both national and local, financially interested in the growing and packing of fruits and vegetables, have greatly aided by grants of money the universities and public health laboratories in an intensive study of the different phases of this important problem. Burke [1] had found spores of B. botulinus on fruit and vegetables and the leaves of trees and plants in widely separated sections of California. It has been my privilege to read the manuscript of a monumental work on the distribution of the spores of B. botulinus in nature, by Meyer and Dubovsky, a portion of which appears in the form of several papers in the present issue of this journal. In one of these papers,[2] soils and other samples from many different localities in California, from the higher mountain regions, through the pasture lands of the foothills to the cultivated valleys, were studied, but in no case have many samples of soil been taken from several places within a comparatively restricted area. It is not within the scope of this paper to enter into a discussion of the many interesting features suggested by the work of these authors that such a study would present. The factors involved in the distribution of the two types of B. botulinus in soils, their origin, telluric differences and analogy to the distribution of B. tetani and other soil anaerobes are presented. The authors believe it possible that type A has its origin in the virgin soils of the mountain ranges, is swept down with such soils into the valleys, and that perhaps type B is merely a mutant of type A. The following report of the results

Received for publication, July 26, 1922.

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* This paper confirms and enhances the conclusions drawn in Paper II. It is a great pleasure to include it in the series of publications dealing with the distribution of B. botulinus in nature. K. F. Meyer.

[1] Jour. Bacteriol., 1919, 4, p. 5.

[2] The Distribution of the Spores of B. botulinus in California, this issue.

of some tests of soils for the presence of B. botulinus, which I undertook in the summer of 1921, may be of some interest in connection with their work.

In preparing for this study, some time was spent at the laboratory of the George Williams Hooper Foundation for Medical Research, University of California, where every facility was placed at my disposal for acquiring the technic employed by the workers in their investigations. In order that my results might be comparable to those obtained in that laboratory, the technic thus acquired was adhered to and is fully described by Dubovsky and Meyer[3] in the first paper of their series.

TABLE 1

RESULTS OF AUTHOR'S EXPERIMENTS

1. S. walnut orchard, Carpinteria	10 miles east of Santa Barbara	B. botulinus, type A
2. F. lemon orchard, Summerland	8 miles east of Santa Barbara	B. botulinus, type A
3. G. olive orchard, Summerland	7 miles east of Santa Barbara	B. botulinus, type A
4. A. lemon orchard (twice), Montecito	6 miles east of Santa Barbara	B. botulinus, type A
5. C. vegetable garden, Montecito	5 miles east of Santa Barbara	B. botulinus, type A
6. Chicken droppings uncontaminated by soil (enclosure within above vegetable garden); 3 samples at different times	5 miles east of Santa Barbara	B. botulinus, weak toxin
7. Mixture fresh horse manure from 3 stalls, Bluebird Stables, Montecito	4 miles east of Santa Barbara	Negative
8. MacK. garden	City of Santa Barbara	B. botulinus, type A
9. Mixture wheat, cornmeal, rolled oats, sunflower seed, Union Commission Co.	City of Santa Barbara	Negative (B. welchii infection
10. Walnut orchard, Goleta	6 miles west of Santa Barbara	Negative
11. B. Ranch, bean field	8 miles west of Santa Barbara	B. botulinus, type A
12. Summit Santa Ynez Mountains	About 15 miles east Refugio Pass; elevation about 3,500 feet; sample taken (away from any trail) in thick underbrush	B. botulinus, type A

The work described in this paper embraces a study of a few soils taken from a narrow strip of coast line about 18 miles long in Santa Barbara County, Calif., from Carpinteria on the east, through the city of Santa Barbara to the Bishop Ranch on the west. This strip averages about a mile and a quarter in width and is backed by the abruptly rising mountains of the Santa Ynez Range. The soils varied from light, sandy and rich loam to heavy clay.

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[3] An Experimental Study of the Methods Available in the Enrichment, Demonstration and Isolation of B. botulinus, this issue.

Some preliminary studies were first undertaken with 3 strains of B. botulinus and a soil sample from the Yosemite Valley sent me by Dr. K. F. Meyer. I was able to confirm his findings for this soil (B. botulinus, type A isolated) as well as for a soil sample taken by him personally from an orchard near Santa Barbara. Each of my samples was made up of a mixture of soil from the surface to a depth of about 6 or 8 inches, often from a gopher mound, and was taken with the usual aseptic precautions and placed in containers sterilized at 170 for 2 hours.

My results are given in table 1.

It is to be noted that the toxin from the cultures of chicken droppings, though giving definite symptoms of B. botulinus poisoning in experimental animals, was too weak to type with antitoxin neutralization tests. Evidence has been given [3] that in such cases few spores exist in the material examined.

Cultures or tests were made of most of these soils several times, as severe tetanus symptoms often developed in the inoculated animals before the B. botulinus symptoms. In some cases, a protective dose of antitetanic serum was also given.

During the latter part of these experiments, liver was added to the beef heart peptic digest broth mediums, which gave a stronger toxin of B. botulinus, thus largely obviating the difficulty with the tetanus toxin.

It has been shown by this investigation that the cultivated soils of a narrow strip of coast line in Santa Barbara County, Calif., are heavily contaminated with the spores of B. botulinus, type A, as well as with those of B. tetani and that the virgin soil from the mountain range behind this strip of land also contains the spores of B. botulinus, type A.

THE DISTRIBUTION OF THE SPORES OF B. BOTULINUS IN THE UNITED STATES. IV *

K. F. MEYER AND BERTHA J. DUBOVSKY

From the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco

It has been customary to consider California an endemic focus of botulism and the prevention of this intoxication a problem confined to the Pacific Coast States. Such a view is generally held in the East and Middle West, and statements have from time to time been issued which tend to absolve the territories east of the Rocky mountains and place the odium of botulism squarely on California. In a recent paper [1] it has been shown that this apparent prevalence of botulism in the last named state is in all probability due to the interest this disease has received at the hands of a number of workers, as a result of which data have been unearthed and analyzed which have thus far not focused attention in the other states of the Union. Furthermore, an unbiased observer will have noted that botulism is an acquisition of recent years and is closely associated with the use of home or commercially canned food products. During the war, economic reasons prompted the adoption of home canning procedures, while later prosperity on one side and domestic labor difficulties on the other led to the extensive consumption of commercially preserved, "ready to serve" foods of plant and animal origin. Since 1912, eighty-seven outbreaks of human and animal botulism have been caused by food products grown and packed in 20 states, exclusive of California. In this connection the following states and outbreaks should be mentioned: Colorado (3 human and 3 fowl), District of Columbia (1 fowl), Florida (1 human), Idaho (3 human), Illinois (1 human, 1 fowl and 1 horse), Indiana (1 human and 1 fowl), Iowa (1 human), Kentucky (1 mule), Main (1 human), Massachusetts (3 human), New Jersey (1 human and fowl), New York (4 human), Nevada (5 fowl), Ohio (2 human), Oregon (4 human and 2 fowl), Pennsylvania (1 human), Texas (1 human), Washington (14 human and 23 chicken), Wisconsin (1 human) and Wyoming (1 human).

Received for publication, July 26, 1922.

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* This study was aided by grants from the National Canners Association, the Canners League of California and the California Olive Association.

[1] The Epidemiology of Botulism, to be published in a Bulletin of the U. S. Public Health Service.

This summary permits one conclusion: The spores of B. botulinus must be widely distributed in the United States. In order to confirm this, however, an examination of soil, vegetable and manure samples procured in every state in the Union was undertaken. It is proposed in this paper to summarize these studies. It will be shown that no territory within the boundaries of the United States can justifiably claim immunity from B. botulinus. Absence of human or animal botulism from a number of states cannot be attributed to the non-existence of the spores of the causative organism in the soil, but is probably the result of certain other factors, which will be discussed in the analysis of the 1,538 specimens secured for this investigation.

ORGANIZATION FOR COLLECTING AND CULTURE OF THE SAMPLES

The same principles as outlined in the preceding paper dealing with the collection of field specimens in California have been followed in this study. It is self-evident that the cooperation of a number of agencies had to be enlisted in order to make a complete and comparative survey. Dr. H. M. Loomis, Director of Inspection of the National Canners Association, through his field staff in Ohio, Michigan, Illinois, Colorado, Wisconsin, etc., obtained and forwarded valuable samples from the cultivated fields supplying the raw material to important canneries in these states. Dr. C. M. Haring, Director, Agricultural Experiment Station, University of California, solicited and secured the cooperation of a large number of agricultural experiment stations. Sets of at least 20 to 30 specimens were procured through these agencies, namely, one earth sample each from a corral which had been used for cattle during the past year, a pig pen, a poultry yard, a field used for corn, for grain, for clover or as pasture during the past year, several samples of cornstalks, of moldy straw, of decaying vegetation, of bean pods and stalks, of peas, of fresh greens, such as beet tops, spinach, etc., and of roots, such as carrots, beets, etc. These specimens proved valuable from a comparative standpoint and furnished a number of important facts. The study dealing with the distribution of B. botulinus in the state of Washington was based on specimens collected by Dr. J. C. Geiger during his survey on botulismus made in the spring of 1921 at Yakima, Tieton, Toppenish and Yelm. State boards of health, universities and colleges from neighboring states—in particular, Nevada, Utah, Oregon, Arizona and Colorado—frequently submitted samples for examination and rendered valuable assistance in this respect. In order to verify the conclusions reached for California that B. botulinus is an organism of the virgin soil, Dr. J. C. Geiger collected, in September and October, 1921, from 25 to 50 earth samples each from the Glacier and Mt. Rainier National Parks, from the Ozark Mountains in Arkansas, from the foothills near Alexandria in Louisiana, from the foothills near Bath in Maine, from the Blue Ridge Mountains near Washington, D. C., from Mount Baker near Saranac Lake in New York and from the Allegheny Mountains in West Virginia.

It is not only a duty, but a great pleasure, to acknowledge the assistance rendered by the various laboratories, experiment stations, universities and individual staff members who devoted time and energy to the collection of specimens and, through their efforts, enabled the Foundation to make a fairly complete and comparative survey.

The samples were collected in containers which had been sterilized by dry heat at a temperature of not less than 170 C. for 2 hours, or by live steam at a pressure of at least 15 pounds for 30 minutes. When received in the laboratory, extreme precautions were taken to avoid mixture or contamination. Mass cultures were prepared according to the procedures outlined in the first paper of this series; in fact, all cultures were made with heated specimens in beef heart peptic digest liver broth and incubated for 10 days. Crude and unfiltered cultures were tested on guinea-pigs either by subcutaneous inoculation or feeding. The nature and type of the toxin were identified on the same species of animal. In a number of instances, pure cultures were isolated from the enrichment cultures.

Experimental Data

The character of the specimens, the cultural results and the epidemiologic facts thus far determined are reported separately for each state. A geographic, alphabetical presentation has again been chosen to facilitate subsequent reference in the discussion.

Alabama.—In August, 1921, Professor Wright A. Gardner, Alabama Polytechnic Institute, Auburn, Alabama, forwarded 16 specimens: soil from hog pen (2), cow lot (2), oat field (1), clover patch (1), chicken yard (1), corn field (1), vegetable garden (2), onions (1), green corn stalks (1), old corn stalks (1), moldy hay (1), lima beans (1), and decaying vegetables (1). B. botulinus type A was found in the culture prepared with the green corn stalks.

Summary: 10 cultivated soils or animal corrals, 6 vegetables and feeds. Total: 16 with 1, or 6.2%, positive culture; 1 type A.

Arizona.—One human outbreak at Florence, commercially canned beets grown and packed in Ohio.

Miss Jane Rider, Director, State Laboratory, University of Arizona, Tucson, submitted, in February, 1921, one sample of "filaree" hay collected in the southern part of the state and suspected of causing forage poisoning in horses. The culture proved nontoxic.

Arkansas.—In May, 1921, Dr. William L. Bleecker, Bacteriologist, University of Arkansas, College of Agriculture, Fayetteville, furnished 21 specimens: soil from pasture (1), cow lot (1), clover field (1), grain field (1), corn field (1), hog lot (2), poultry yard (1), cow peas (1), radishes (2), spinach (1), soja beans (1), lettuce (1), cornstalk (1), corn fodder (1), rotted straw (1), decaying leaves (2), moldy straw (1), and moldy silage (1).

The rotted straw gave a culture containing the toxin of B. botulinus type B, while the soja beans, decayed leaves and the soil from the hog pen produced toxic cultures, but the nature of the toxin could not be determined by a toxin-antitoxin test.

In November, 1921, Dr. J. C. Geiger collected 20 samples of virgin (?) soil from the Ozark Mountains, Government Reservation, Hot Springs, Ark. Two cultures were weakly toxic, 1 contained B. tetani and 4 produced "malignant edema."

Summary: 8 cultivated soils or animal corrals, 1 weak toxin, 20 virgin soil, 2 weak toxin, 13 feeds, vegetables, 1 type B; 2 weak toxin. Total: 41 with 1 (6), or 2.4 (14.8) % positive cultures.

Colorado.—Three human outbreaks (2 caused by commercially canned string beans or spinach grown and packed in the state of Kansas; 1 home canned beets grown and packed near Pueblo); 3 chicken outbreaks due to home canned vegetables (2 corn, Fort Collins, and 1 string beans, Lyons).

Mr. R. S. Hiltner, Director, Colorado Inspection Service, of the National Canners Association, Denver, procured, in December, 1920, from Brighton (4,970 ft.) and Longmont (4,935 ft.), Colorado, a total of 27 samples consisting of soils (12), tomato plants and roots (4), bean plants, vines and roots (8), pumpkin and cucumber vines and roots (3) were cultures; one culture, prepared from a wax bean plant (East of Longmont), was toxic, but the nature of the toxin could not be determined by a toxin-antitoxin test.

In December, 1921, Inspector M. M. Allison collected 3 sets of soil and plant remnants from farms near Fowler, Colo., in the Arkansas Valley, about 40 miles east of Pueblo. The farms were irrigated by ditches from the Arkansas River. Nine cultures, prepared from soils (3), tomato stems, leaves and roots (2), bean stalks and roots (2) and cucumber vines and roots (2), gave the following results:

Soil, tomato field	B. botulinus, type A.
Soil, bean field	
Bean stalks	
Bean roots	

Tomato roots and vines from cucumber fields produced weakly toxic cultures.

Dr. I. E. Newsom, Department of Pathology, Colorado Agricultural College and Experiment Station, Fort Collins, submitted for examination a specimen of dried crop content of a chicken (Lyons) and 5 samples of moldy alfalfa and dry beet pulp (feed lot, Ault sugar farm near Greeley). The crop specimen contained B. botulinus, type A (for details see Chicken Report 41). In April, 1922, a specimen of home canned corn and the crop content of a chicken, which contained B. botulinus, type A, was received from the same laboratory.

In Sept., 1921, Drs. Oscar I. Kron and F. M. Hayes collected 14 virgin soil samples from Pike's Peak (at an elevation of 11,000 ft.), 5 samples from Cheyenne Canyon, 4 from Mushroom Park, Garden of the Gods, and 4 from the Cave of the Winds. The cultural findings were:

Pike's Peak, 4—B. botulinus, type A; 2 weak toxins.
Cheyenne Canyon, 1—B. botulinus, type A.
Mushroom Park, 1—B. botulinus, type A.
Cave of the Winds, 1—B. botulinus, type A.

Summary: 32 cultivated soils and its products; 1 weak toxin; 9 irrigated soils and its products; 4 type A; 2 weak toxins; 27 virgin mountain soil; 7 type A; 2 weak toxins.

Total: 68 samples with 11 (16), or 16.1 (25.0) % positive cultures.

One crop specimen (home canned string beans) type A; 1 home canned corn and 1 crop specimen, type A.

Connecticut.—In Sept., 1921, Mr. C. J. Mason, Instructor in Bacteriology, Connecticut Agricultural College, Storrs, collected and submitted for examination, 9 samples of soil (poultry yard, pig pasture [2], grass field [2], clover field, cattle corral, pig pen, corn field) and 15 vegetable specimens (beets, potatoes, carrots [2], peas, musty straw, beet greens, corn stalks, turnip tops, decayed tomatoes, cucumbers, bean stalks, bean pods, musty hay, mangel tops). The beets furnished a type A, B. botulinus, while the potatoes, musty straw, corn stalks, decayed bean stalks and tomatoes gave weakly toxic cultures.

Summary: 9 cultivated or manured soils, 15 vegetables, feed, etc.—1 type A, 5 weak toxins. Total: 24 samples with 4.1 (25) % positive cultures.

Delaware. — Mr. F. C. Blanck, Director of Inspection, National Canners Association, Easton, Md., collected and forwarded, in December, 1920, from different points in Delaware, 6 samples of soil (lima bean, tomato, red pepper, string bean, bush bean and sweet potato fields) and 12 specimens of leaves and roots from the fields mentioned. The following cultures were toxic:

Soil, husks and leaves from string bean field, 2 B. botulinus, type B.
Soil, tops and leaves from tomato field; 2 B. botulinus, type B.
Lima beans and bush bean husks, weak toxins.

Summary: 6 cultivated soil, 2 type B. 12 plant products, 2 type B and 2 weak toxins.

Total: 18 samples with 4 (16), or 22.2 (33.3) % positive cultures.

District of Columbia.—One chicken outbreak, due to corn, no earth samples.

Florida.—One human outbreak at Tampa due to home preserved ham.

In November, 1921, Dr. J. C. Geiger collected, near Miami, 20 samples of unfertilized soil. One culture contained a weak toxin. At the same time, Dr. W. G. W. Ells, Assistant Plant Pathologist, Agricultural Experiment Station, University of Florida, Gainesville, collected and forwarded 25 duplicate samples of soils and decayed plants from the vicinity of the experiment station. Cultures were made from soil from cattle corral, horse corral, cane field, citrus orchard, garden, corn field, hog lot, chicken run, muck soil, peat, clay, barren field, dirt road, edge of pond, from under yellow pine tree, shrub land, potato land, Bermuda sod, meadow (Natal grass), cherry laurel hedge, virgin soil from hammock, oak and hickory, yellow pine land and large oak land and decomposed roots and leaves. Eight cultures were positive, namely:

Decomposed roots and leaves, 2—B. botulinus, type B.
Soil from citrus orchard, 1—B. botulinus, type B.
Soil from meadow, Natal grass, 1—B. botulinus, type B.
Muck soil, 1—B. botulinus, type B.
Soil from edge of a pond, 1—B. botulinus, type B.
Soil from ordinary, poor blackjack shrub land, 1—B. botulinus, type B.
Soil from cane field, 1—B. botulinus, type A.

Summary: 43 soils from diverse sources, 5 type B, B. botulinus, 1 type A, 1 weak toxin; 2 decomposed plants, 2 type B.; 45 with 8, or 17.7% positive cultures.

Georgia.—In May, 1921, Dr. B. B. Higgins, Botanist, Georgia Experiment Station, Experiment, Ga., collected and submitted for examination 20 samples of soils and its products: soil from pasture, cow lot, corn stubble, clover land, chicken yard, hog lot and virgin soil, moldy hay (2), bean pods and stalks, beans and vine, cabbage (2), peas, cornstalk, onions, daffodil bulbs, turnips, English garden peas, beet root. Five cultures gave relatively strong toxins:

Moldy hay, 1—B. botulinus, type B.
Bean pods and stalks, 1—B. botulinus, type B.
Beans and vines, 1—B. botulinus, type B.
Decayed cabbage, 1—B. botulinus, type B.
Turnips, 1—B. botulinus, type B.

Summary: 7 soil samples, all negative; 13 vegetables and feeds, 5 type B.

Total: 20 samples with 5, or 25%, positive cultures.

Idaho.—Three human outbreaks (1 locally grown and home canned asparagus, Boise, 1 locally grown and home canned greens and turnip tops, Cambridge, and 1 locally grown and home canned corn, Weiser).

In November, 1921, Professor William M. Gibbs, head of the Department of Bacteriology, College of Agriculture and Agricultural Experiment Station, University of Idaho, Moscow, collected and forwarded for examination 32 samples of soils and vegetables and feeds: soil from turnip field, chicken yard, cattle pasture, manure, pea field, corn field, sheep corral, cattle corral (2), pig pen, orchard, grain lot, subsoil excavation, virgin soil (3), turnip tops (2), grass from pasture, corn stalk, peas and roots, corn, decomposed silage, larch leaves (Arboretum), cabbage, apples, pears and cucumbers rotting on ground, beet tops, beets dug from ground, chard and parsnips dug from ground.

Three cultures were highly toxic, while 3 produced symptoms, but could not be identified. The following samples furnished these cultures:

Virgin soil under larch growth, B. botulinus, type A.

Cucumber rotting on ground, B. botulinus, type A (isolated).

Beet tops, beets still growing, B. botulinus, type B.

Soil from turnip field, cornstalk and beets, dug from ground gave weak toxins.

In February, 1922, Dr. W. R. Hamilton, Weiser, sent a jar of locally grown and packed home canned corn belonging to the same lot, which caused the death of one child at Weiser, Report 99. The corn was toxic and B. botulinus, type A, was isolated.

Summary: 15 soil samples, 1 virgin soil, type A; 18 vegetables and feeds and home canned plant products, 2 type A and 1 type B, 3 weak toxins.

Total: 33 samples with 4 (7), or 12.1 (21.2)%, positive cultures.

Illinois.—One incompletely proved human outbreak at Chicago, one chicken outbreak, due to garbage, one outbreak among horses caused by ensilage.

The Hoopeston Canning Company submitted, in December, 1920, 2 samples of soil collected from heavily manured corn fields and 2 specimens of sweet corn roots; 8 cultures were prepared and one of each soil specimen contained a weak toxin, producing symptoms and death in 10 days.

The Gibson Canning Company, Gibson City, furnished, in December, 1920, one soil sample and 5 specimens of sweet corn stalks, husks, leaves, etc. One culture prepared with the corn stalk contained a toxin which was fatal to a guinea-pig on the twenty-sixth day.

Mr. David M. Morgan of Mound City forwarded 5 samples of soil (sweet corn, tomato, sweet potato, hominy, corn and pumpkin fields) and 10 speciments of roots, leaves, vines, etc., collected from the same areas in December, 1920. Ten specimens of the cultures prepared with the sweet corn and sweet potato leaves contained B. botulinus, type B.

The Interrieden Canning Company, Grayslake, forwarded 5 soil specimens and 7 samples of corn leaves, husks, pea vines, roots and leaves collected on their farms, in December, 1920. The cultures prepared with these specimens were all nontoxic.

The George E. Stocking Canning Factory, Rochelle, supplied 2 specimens of soil derived from a pea field together with some leaves of a well cured stack. The samples furnished nontoxic cultures.

Summary: 14 soils from pea, corn, tomato, etc., fields, 2 weak toxins; 25 leaves, husks, vines, etc., 2 type B, B. botulinus, and 1 weak toxin.

Total: 39 samples with 2 (5), or 5.1 (12.8) %, positive cultures.

Indiana.—Two human outbreaks (one due to locally grown and home canned string beans and one due to commercially packed spinach from California, at Kendallville).

Mr. John P. Street, Director of Indiana Inspection Service, National Canners Association, Indianapolis, procured 1 sample of soil and 2 specimens of roots and stems from a tomato field at Paoli, and 3 similar specimens from Red Key. These samples furnished nontoxic cultures.

Dr. L. P. Doyle, Purdue University, Agricultural Experiment Station, Lafayette, collected and submitted for examination 7 samples of soil from cattle pen, pig pen, poultry yard, corn field, wheat field, clover field and pasture, and 15 specimens of vegetables and plants: corn stalk, decaying vegetables (2), straw, moldy straw, beanstalks, bean pods (2), pea vine, swiss chard, beet top, turnips, beets, carrots and radishes. The following cultures were toxic:

Soil corn field, 1—B. botulinus, type B.
Soil wheat field, 1—B. botulinus, type B.
Soil clover field, 1—B. botulinus, type B.
Soil pasture, 1—weak toxin and B. tetani.
Bean stalks and pods, 2—B. botulinus, type B, 1 weak toxin.
Radishes, 1—weak toxin.
Swiss chard, B. tetani.

Summary: 9 soils from cultivated fields and pasture, 3 type B, 1 weak toxin and B. tetani; 19 vegetables and feeds, 2 type B, 2 weak toxin and B. tetani.

Total: 28 samples with 5 (8), or 17.8 (28.4) %, positive cultures.

Iowa. — One unproved human outbreak, due to home prepared ham and salted pork, at Sioux Rapids.

Mr. William H. Harrison, Director of the Iowa Inspection Service, National Canners Association, collected and forwarded, in December, 1920, one sample of soil and one specimen each of corn leaves, husks and roots on a farm near Altoona. Similar sets of specimens were taken at Grimes (about 14 miles northwest of DesMoines) and at Vinton.

Dr. Charles Murray, Division of Veterinary Medicine, Iowa State College, Ames, collected, in September, 1921, and forwarded for study 8 samples of soil (hog yard, horse corral, cattle corral, poultry yard, corn field, clover field, oat field, pasture) and 14 specimens of vegetables and feeds: oats (2), beets, carrots, turnips, beans and pods, decayed straw, cabbage, radish roots and tops, moldy hay, corn stalks, decayed vegetables (2).

One sample of oats gave a culture containing B. botulinus type A, while those prepared from the radish roots and carrots were weakly toxic.

Summary: 11 cultivated soils, all negative; 23 vegetables and feeds, 1 type A, 2 weak toxins.

Total: 34 samples with 1 (3), or 2.9 (5.8) %, positive.

Kansas.—Mr. H. R. Baker, Department of Bacteriology, Kansas State Agricultural College, Manhattan, collected and forwarded, in May, 1921, 10 samples of soil (horse corral [2], cow pasture, cow corral, pig pen, sheep corral, poultry yard, grain field, clover field and corn field fertilized with sheep manure) and 20 specimens of vegetables and feeds: bottom of a Sudan grass straw stack, decayed straw (2), corn stalks, rotted, frost bitten alfalfa, young green mangels, young turnips, decayed leaves in barnyard, spinach, straw, beets, string beans, carrots, radishes, beet tops, pea stalks, decayed vegetation, and bean stalks and lettuce (2).

Beets bought on the market, B. botulinus, type A.
String beans bought on the market, B. botulinus, type A.

Summary: 10 cultivated or manured soils, all negative; 20 vegetables and feeds, 2 type A.

Total: 30 samples with 2, or 6.6%, positive cultures.

Kentucky.—One outbreak among mules, due to ensilage prepared in Carroll County.

Dr. A. J. Steiner, Department of Veterinary Science, Agricultural Experiment Station of the College of Agriculture, University of Kentucky, Lexington, collected and submitted, in June, 1921, 7 samples of soil (hog pen, dairy pasture, manure and soil from cow run, chicken run, corn field [2], rye field) and ten specimens of vegetables and feeds: asparagus roots, potato, wheat heads, lettuce, spinach, onions, beets, corn stalks, soja beans and radishes. The following specimens furnished highly toxic cultures:

Soil from hog pen on college farm (has been used as such for many years, B. botulinus, type B.

Soil from dairy pasture, B. botulinus, type B.

Soil from field that contained clover last year, corn this year, B. botulinus, type B.

Soil from plot which has been planted in corn for the last seven years, B. botulinus, type B.

Radishes, onions, beets and lettuce from Dr. D.'s garden, 4 B. botulinus, type B.

Potato from College farm garden, B. botulinus, type B.

Wheat heads from College farm, B. botulinus, type B.

Corn stalks from Experiment Station Farm, B. botulinus, type A.

Soja beans from Experiment Station Farm, B. botulinus, type B.

Summary: 7 cultivated or manured soils, 4 type B; 10 vegetables and feeds, 7 type B, 1 type A.

Total: 17 samples with 12, or 70.5%, positive cultures.

Louisiana.—In March, 1921, Dr. Harry Morris, Bacteriologist, College of Agriculture and Agricultural Experiment Station, Louisiana State University, Baton Rouge, collected around the University Campus and Experiment Station 8 samples of soil (cattle lot, hog lot, chicken yard, oat field, sugar cane field, pasture, corn field, alfalfa field) and 20 specimens of plant products: mustard, beets, decaying rice straw (2), moldy soja bean hay, lettuce, pea pods and stalks, soja beans, decaying onions, beet tops, green beans, decaying sweet potatoes, decaying leaves, carrot tops, carrots, decaying cabbage leaves, corn stalks (2), husks and corn. The following cultures were toxic:

Corn stalks, B. botulinus, type A.

Moldy soja bean hay, B. botulinus, type A.

Lettuce, beet tops, green beans and corn furnished weakly toxic cultures.

In November, 1921, Dr. J. C. Geiger collected in the foothills 5 miles north of Alexandria, 20 samples of soil from cut over timberland used for grazing purposes. The cultures were all nontoxic.

Summary: 8 cultivated soils, all negative; 20 timberland, used for grazing, all negative; 20 vegetables and feeds, 2 type A, 4 weak toxins.

Total: 48 samples with 2 (6), or 4.1 (12.5) %, positive cultures.

Maine.—One human outbreak due to commercially prepared ham at a summer resort.

Miss F. L. Chandler, Department of Bacteriology and Veterinary Science, University of Maine, Orono, collected and forwarded, in September, 1921, 7 samples of soil (corn field, clover field, hen yard, cow yard, pig pen, pasture, grain field) and 14 specimens of vegetables and plants (cucumbers, lettuce, decaying vegetables, tomatoes, cabbage, beets and tops, beans, carrots, rape, corn stalks, moldy hay, turnips, spoiled silage, pea pods and stalks). The following cultures were highly toxic:

Lettuce, cabbage, moldy hay, pea pods and stalks, 4 B. botulinus, type A.

Beans and turnips, 2 B. botulinus, type B.

In October, 1921, Dr. J. C. Geiger collected from the foothills (uncultivated section) near Bath, Lewiston, Augusta and Poland Springs 20 samples of virgin forest soil. Four cultures made from red soil were highly toxic and contained B. botulinus, type A.

Summary: 7 cultivated soil, all negative; 20 virgin forest soil, 4 type A; 14 vegetables and feeds, 4 type A, 2 type B.

Total: 41 samples with 10, or 24.3%, positive cultures.

Maryland.—Mr. F. C. Blanck, Director of Inspection, National Canners Association, Easton, collected and forwarded, in December, 1920, 2 samples of soil (tomato and corn fields) and 4 specimens of stems and roots procured from the same fields. The cultures prepared from the soil of the corn field contained B. botulinus, type A, while those made with soil of the tomato field and tomato roots were not sufficiently toxic to permit identification.

In February, 1921, cultures were made of 4 samples of hog manure obtained at Perryville, with negative results.

Dr. J. C. Geiger collected 20 samples of virgin soil about 40 to 50 miles from Washington, D. C., in the foothill region of the Blue Ridge Mountains. Eleven cultures contained B. botulinus, type B, 2 type A and B, and 1 B. botulinus, type B, and B. tetani.

Summary: 2 cultivated soil, 1 type A, 1 weak toxin; 20 virgin soil, 11 type B, 2 type A and B, 1 B. tetani and B. botulinus; 4 vegetables, 1 weak toxin; 4 hog manure, all negative.

Total: 30 samples with 15 (17), or 50 (56.6) %, positive cultures.

Massachusetts.—Three human outbreaks (one due to minced chicken, one due to locally prepared blood sausage, at Lowell, one cause unknown).

Mr. J. Raymond Sanborn, Department of Microbiology, Massachusetts Agricultural College, Amherst, collected and forwarded, in April, 1921, 5 samples of soil (clover field, poultry yard, cow corral, pasture land and corn field) and 12 specimens of vegetables and feeds: parsnips, cabbage stubs, moldy hay (2), corn stalks (2), carrots, dandelion, lettuce, beans, soja beans and decaying corn stalks. The soil from the pasture land contained B. botulinus, type B, while the parsnips and beans produced weak toxins.

Summary: 5 cultivated and manured soils, 1 type B; 12 vegetables and feeds, 2 weak toxins.

Total: 17 samples with 1 (3), or 5.8 (17.0) %, positive cultures.

Michigan.—Three human outbreaks due to olives and spinach commercially packed in California.

Mr. Frank Gerber, of the Fremont Canning Company, collected, in November, 1920, and forwarded through Mr. A. B. Todd, Director of Inspection for Michigan, National Canners Association, 24 samples of soils (6 specimens each from pea fields, corn fields, Michigan bean fields, and cabbage fields) and 24 specimens of pea vines, corn husks and white bean vines and roots.

One sample each of the corn husks, white bean vines, pea vines and soil of corn garden produced weak toxins.

In July, 1921, Mrs. Zae Northrup Wyant, Research Associate in Bacteriology, Michigan Agricultural College, East Lansing, collected and submitted for examination 28 samples of soil: newly cultivated bean field, bean field, oat field, corn garden, pea garden, potato garden, clay soil from garden, soil from garden (2), soil from newly plowed field, soil from under mulch on strawberries, soil from barnyard, soil from vineyard, soil from 7 pens poultry house, pig pen, yard, alfalfa field, pea patch, cucumber patch, barnyard, timothy field, corn field, bean field, potato field, wheat field, garden soil, pole bean garden, poultry ranch, 5 specimens of silage (from bottom of Bacteriology barn silo); 5 samples of feeds (lettuce, new timothy and clover hay, dried beet pulp, ear corn, corn flakes) and 14 samples of manure and compost. The following cultures were positive:

Silage northwest silo, Dairy barn, 2 feet deep only; B. botulinus, type B (48 hour toxin).

Soil from underneath mulch on strawberries, College Farm, B. botulinus, type B.

Soil and bedding from pig pen and yard, west end of barn, weak toxin.

Chicken manure (Wyant); B. botulinus, type A.

In September, 1921, Dr. J. C. Geiger obtained 10 samples of virgin soil from wooded areas (20 to 30 miles from Detroit) and 10 samples of garden soil from the Tuberculosis Farm near Detroit. Two cultures prepared with garden soil contained B. botulinus, type B, and 2 other cultures B. tetani.

Summary: 82 fertilized or manured soils, 3 type B, 2 weak toxins, 2 B. tetani; 10 virgin soil, all negative; 5 silage, 1 type B; 29 vegetables and feeds, etc., 3 weak toxins; 14 manure and compost, 1 type A.

Total: 120 samples with 5 (10), or 4.1 (8.3) %, positive cultures.

Minnesota.—The Minnesota Valley Canning Company, Le Suer, submitted in December, 1920, 4 samples of soil (corn ground [2] and pea fields [2]) and 4 specimens of corn husks, pea plants and roots. The cultures prepared with these samples were all negative. Additional samples of soil (4) and plant products (4) were sent from other farms of the same company. The cultures were nontoxic.

Mr. C. D. Geidel, Director of the Minnesota Inspection Service, National Canners Association, St. Paul, forwarded in January, 1921, 6 samples of soil (3 different farms) and 4 specimens of corn roots and corn leaves collected by the Barr Pickling and Preserving Company of St. Cloud. One culture of the upland soil, Barr Farm, contained B. botulinus, type A, while 2 other soil specimens produced weak toxins.

In October, 1921, Dr. J. C. Geiger collected 10 samples of virgin soil from Minnehaha Creek in the vicinity of Minneapolis and 10 samples of soils from vegetable gardens. One culture of garden soil contained B. botulinus, type A and one specimen of virgin soil produced a weak toxin. Two garden soils contained B. tetani.

Summary: 24 cultivated or manured soils; 2 type A and 2 weak toxins; 10 virgin soil, 1 weak toxin; 12 roots, leaves, vegetables, etc., all negative.

Total: 46 samples with 2 (5), or 4.3 (10.8) %, positive cultures.

Mississippi.—Dr. Charles F. Briscoe, Bacteriologist, Mississippi Agricultural and Mechanical College, Agricultural College, collected and forwarded, in May, 1921, 6 samples of soil (cow stable, pig pen, poultry pen, clover field, unknown [2]) and 13 specimens of vegetables, feeds, roots, etc.: decaying vegetation (2), bean pods and stalks, moldy hay (2), pea pods and stalks, corn stalks, turnip roots, radish tops, beet roots, carrot roots, fresh lettuce and beet tops. The following cultures were toxic:

Decaying vegetation, B. botulinus, type B.

Corn stalks, B. botulinus, type B.

Turnip roots, B. botulinus, type B.

One sample each of soil, moldy hay and carrot roots gave weakly toxic cultures.

Summary: 6 cultivated or manured soils, 1 weak toxin; 13 vegetables, feeds, etc., 3 B. botulinus, type B, 2 weak toxins.

Total: 19 samples with 3 (6), or 15.7 (31.5) %, positive cultures.

Missouri. — Prof. William A. Albrecht, Department of Soils, College of Agriculture, University of Missouri, Columbia, collected and forwarded, in March, 1921, 7 samples of continuously cropped and manured soil (blue grass pasture, hog lot, rye field, cattle lot, chicken yard, corn lot, clover field) and 7 specimens of vegetables, compost, etc. (ensilage [2], corn stalks, decaying blue grass hay, leaf compost, blue grass manure, fresh lettuce) and jars of spoiled home canned beans. The following cultures were toxic:

Soils from blue grass pasture, hog lot and corn field, 3 B. botulinus, type B.

Ensilage fresh collection, 1 B. botulinus, type B.

Decaying blue grass hay, 1 B. botulinus, type B.

Manure blue grass, 1 B. botulinus, type B.

Home canned beans (nontoxic brine), 1 B. botulinus, type A.

One of us (B. J. D.) collected, in July, 1921, near the Campus of the University of Missouri, 10 samples of soil (street, driveway, clay soil, top soil, turned up soil near pond, walk near dairy), wheat (2), and manure (2), and 7 specimens of vegetables, feeds and manure (decomposed hay [2], wheat [3], manure [2]). The following cultures were toxic:

Soils, near pond, near dairy, wheat field (2), 4 B. botulinus, type B.

Two soil samples and one specimen of wheat gave weak toxin.

In June, 1921, the laboratory received from Peruque, two small wild ducks. The cadavers were badly decomposed, but coccidia were found in the liver and intestines. Cultures were taken from a composite sample of the intestinal tract and B. botulinus, type B was demonstrated in these specimens.

Summary: 17 soils continuously cropped and manured, etc., 7 type B and 2 weak toxins; 14 vegetables, ensilage, manure, 3 type B and 1 weak toxin; 1 intestines of wild ducks, 1 type B; 2 home canned string beans, 1 type A.

Total: 34 samples with 12 (15), or 35.2 (44.1) %, positive cultures.

Montana.—One human outbreak at Java, due to stuffed ripe olives grown in California.

Dr. D. B. Swingle, Department of Botany and Bacteriology, Agricultural Experiment Station, University of Montana, Bozeman, collected and forwarded, in September, 1921, 9 samples of soil and dirt (rabbit yard, clover field, corn field, chicken pen, pig corral, sheep pen, horse pen, grain field, duck pen) and 16 specimens of vegetables, manure, silage, etc. (carrots, moldy alfalfa, beans, decayed beets, rutabaga, peas, summer squash, lettuce, corn silage, tomatoes, crab apple, cabbage leaves, parsley, spinach and cucumbers, cow manure). The following cultures were toxic:

Carrots, lettuce, corn silage, 3 type A.

Moldy alfalfa, 1 type A and B.

Dirt, horse pen, 1 type B.

Parsley, green tomatoes, rutabaga and summer squash, 4 weak toxins.

Dr. J. C. Geiger collected, in September, 1921, 41 samples of soil from 3 different areas in Glacier National Park. The cultures gave the following results:

Area 1: West side of Continental Divide along Lake Ellen Wilson and Gunsight Pass—12 samples, 5 type A.

Area 2: East side of Continental Divide, foot of Swift Current Pass and Mt. Wilbur—16 samples, 2 type A and 2 type B, 2 weak toxins.

Area 3: Foothills of eastern side of Continental Divide (Midvale Creek and Squaw Mountain)—13 samples, 2 type A and 5 weak toxins.

Summary: 9 soils or dirt from manured places, 1 type B; 41 virgin mountain soils, 9 type A, 2 type B; 7 weak toxins; 16 vegetables, feeds, etc., 3 type A, 1 type A and B, 4 weak toxins.

Total: 66 samples with 16 (27), or 24.2 (40.9) %, positive cultures.

Nebraska.—Dr. L. Van Es, Department of Animal Pathology and Hygiene, University of Nebraska, Lincoln, forwarded, in May, 1921, 12 samples of soil (poultry yard, cow yard, sheep yard, cattle yard, hog yard, wheat field, corn field, field No. 8, small bull pasture, hog yard (serum plant), horse yard, alfalfa field) and 11 specimens of vegetables, feeds, etc. (bottom of old hay stacks [2], corn stalks, corn silage, lettuce, alfalfa, decayed cabbage stalks, old manure pile [2], dandelions, old beets from root cellar). The following cultures were toxic:

Old manure pile (Havelock), 1 B. botulinus, type A.

Dandelions, 1 B. botulinus, type A.

Soil, poultry yard, hog yard, wheat field, alfalfa field, gave weak toxins.

Summary: 12 cultivated or manured soils, 4 weak toxins; 11 vegetables and manure pile, 2 type A.

Total: 23 samples with 2 (6), or 8.6 (26.0) % positive cultures.

Nevada.—Five chicken outbreaks, due to home canned string beans (1), moldy potatoes (2), spoiled sour milk (1) and spoiled canned goods (1).

Dr. J. C. Geiger brought, in December, 1920, 3 samples of field soil and of garden soil from the vicinity of Reno and 2 samples of garden and 2 of desert soil from Hastings. One sample of garden and one of desert soil produced weakly toxic cultures. The remainder was nontoxic.

Mr. G. D. Delprat, Jr., collected in Sage County near the California border 3 samples of virgin soil. Two contained B. botulinus, type A.

Dr. L. R. Vawter, University of Nevada, forwarded, in December, 1921, 11 samples of soil. The cultures gave the following results:

Virgin soil along road through secret pass in the Ruby Mountains (7,000 feet)—1 type A (south of Elko).

Sage brush bench, north of Elko, used as a range for cattle and sheep—1 type A.

Creek bed soil, Gardnerville—1 type A.

Soil alfalfa field, Truckee Valley, irrigated 8 miles south of Reno—1 type A.

Virgin sage brush soil at foot of Mt. Rose (20 miles southwest of Reno)—1 type A.

Lake bed soil, bank of Little Washoe Lake, in Washoe Valley—1 type A.

Garden soil in Reno (avian botulism), hayfield, and river bank adjoining Humboldt River, Elko, garden soil in Gardnerville and Steamboat Springs, Geyser Basin—all negative.

Summary: 5 field soil, 1 B. botulinus, type A; 8 garden soil, 1 B. botulinus, type A, 1 weak toxin; 4 desert and sage brush, 2 B. botulinus, type A, 1 weak toxin; 8 virgin soil, 5 B. botulinus, type A.

Total: 25 samples with 9 (11), or 36.0 (44.0) %, positive cultures.

Several isolations of B. botulinus, type A and B from the livers of cattle by Dr. L. R. Vawter should be added.

New Hampshire.—Prof. Mabel Brown, New Hampshire Agricultural Experiment Station, Durham, collected and forwarded 6 samples of soil (cattle corral, poultry yard, oat field, corn field, pig pen, clover field) and 8 specimens of vegetables and feeds (radishes, bean stalk, pea stalk, beets, decaying leaves, lettuce, hay and pea pods).

The sample of hay contained B. botulinus, type B; the pea stalk gave a weak toxin.

Summary: 6 cultivated or manured soils, all negative; 8 vegetables and feeds, 1 B. botulinus, type B, 1 weak toxin.

Total: 14 samples with 1 (2), or 7.1 (14.2) %, positive cultures.

New Jersey.—One human outbreak, due to home canned string beans, at Newark.

Dr. J. H. McNeil, Chief, Bureau of Animal Industry, Department of Agriculture, Trenton, forwarded, in February, 1921, 5 samples of soil collected from salt meadows near Toms River and Osbornville, eastern section of the state. One sample from J. Farm, one from Westervelt and one from Johns Farm contained B. botulinus, type B.

Dr. Fred Boerner, Jr., Bureau of Animal Industry, Pennsylvania Department of Agriculture, Philadelphia, collected and forwarded, in February, 1921, 20 samples of soil and feeds from 8 different localities. The following results were obtained with the cultures:

Soil and sod from salt meadow, Cape May, 2 B. botulinus, type B.
Soil, mixture of hay (1), hay, Swainton, 1 weak toxin.
Soil and corn, South Dennis, all negative.
Soil (2), forage alfalfa, corn, woodbine, all negative (4).
Soil, corn, salt hay, corn stover, near Goshen, all negative.
Soil, Dias Creek, negative (1).
Soil, feed mixture, corn stover, salt hay, all negative (4).
Soil, Rio Grande, 1 weak toxin.

Two samples of soil collected from tomato field near Camden were cultivated, in February, 1921, and found to be free from B. botulinus spores.

Dr. F. G. Steinbach of Wildwood submitted, in October, 1921, 3 samples of soil (H. and F. Farms) one of manure (1), and 4 of feeds (alfalfa, moldy bread, corn stubble and stover). The cultures gave the following results:

Soil H. farm, 1 B. botulinus, type A.
Soil F. farm, 1 B. botulinus, type B.

Summary: 20 meadow soils and sod from salt farms, 6 type B, 1 type A, 2 weak toxins; 1 manure, negative; 15 feeds, hay corn, etc., all negative.

Total: 36 samples with 7 (9), or 19.4 (25) %, positive cultures.

New Mexico.—Mr. Harold Gray, State Engineer, Santa Fe, forwarded, in December, 1920, 8 samples of soil supposedly collected in various sections of the state. The cultures prepared from these samples were all nontoxic.

New York.—Five human outbreaks, one in New York City, due to ripe olives grown and packed in California, one to home prepared cottage cheese in the western part of the state, one to home canned corn, one to commercially packed spinach and one to unknown food products, the last two in New York City.

S. K. Farrar, Assistant Director of Inspection, National Canners Association, Rochester, forwarded, in January, 1921, 3 samples of soil (Red Creek and Oswego) and 4 specimens of cabbage leaves, corn husks and pea vines. The cultures gave the following results:

Soil, Oswego, B. botulinus, type A.
Cabbage from field near Red Creek, B. botulinus, type A.
Peavine, weak toxin.

Dr. George A. Stock, Medical Examiner, U. S. Veterans Bureau, forwarded, in October, 1921, 24 samples of virgin soil collected from Mount Baker, which is in the vicinity of Saranac Lake. Four samples contained B. botulinus, type A. 1 gave a weak toxin and 3 contained B. tetani.

Summary: 3 cultivated and manured fields, 1 type A; 24 virgin soils, 4 type A, 1 weak toxin; 4 vegetables, etc., 1 type A, 1 weak toxin.

Total: 31 samples with 6 (8), or 19.3 (25.8) %, positive cultures.

North Carolina.—Dr. F. A. Wolf, Department of Botany, North Carolina Agricultural Experiment Station, West Raleigh, collected and forwarded, in

April, 1921, 12 samples of soil (cow pen, vineyard, garden, cattle and sheep pasture, oat field, pig pen, apple orchard and chicken pen, clover field and corn field, sheep pen and horse pen) and 8 specimens of vegetables and feeds (cabbages, peas, lettuce, chard, onions, corn stalks, beans and radishes). The following cultures contained weak toxins, which could not be identified by toxin-antitoxin tests: soil from vineyard, garden, cattle pasture, radishes, chard and onions.

Summary: 12 cultivated soils, 3 weak toxins; 8 vegetables, 3 weak toxins.

Total: 20 samples with 6, or 30%, toxic cultures.

North Dakota.—Dr. A. F. Schalk, North Dakota Agricultural College, collected and forwarded, in May, 1921, 7 samples of soil (cattle corral, poultry yard, corn field, clover field, pig pen, pasture, grain field) and 7 specimens of vegetables and feeds (radishes, moldy straw, forage, corn stalks, decaying roots [2] and lettuce).

The culture prepared with the forage contained B. botulinus, type A, while those made from the moldy straw and corn stalks were weakly toxic.

Summary: 7 cultivated soils, all negative; 7 vegetables and feeds, 1 type A, and 2 weak toxins.

Total: 14 samples with 1 (3), or 7.1 (21.4) %, positive cultures.

Ohio.—Two human outbreaks, at Canton, one due to ripe olives packed in California and one at Canaan, due to spinach.

The Sears Nichols Canning Company at Chillicothe submitted, in June, 1920, 2 samples of beets and soil. Six cultures were prepared and found to be nontoxic. In October, 1920, the same company forwarded 5 samples of soil (beet field [2], lima bean field, sweet corn field and spinach field) and 7 specimens of beets, lima beans, green corn and raw spinach. A sample of soil from the sweet corn field contained B. botulinus, type B.

Mr. A. M. Wadsworth, Director of Inspection, National Canners Association, Columbus, forwarded, in December, 1920, from Canal Winchester, 6 samples of soil and 8 specimens of vegetables and plant remnants from pea, lima bean and corn fields.

One sample of lima beans from G. B. farm contained B. botulinus, type B.

One sample of corn husks from C. A. farm produced a weak toxin.

M. A. Bates, Assistant Director of Inspection, National Canners Association, Columbus, forwarded, in December, 1920, 5 samples of soil and 8 specimens of vegetables and plant remnants from a cabbage field near Fremont and Bellevue, corn and strawberry fields near Elyria, tomato fields near Madison and corn fields near DeGraff. A culture prepared with cabbage leaves from Bellevue contained B. botulinus, type B and another from Fremont produced a weak toxin.

Summary: 18 cultivated soils, 1 type B; 23 vegetables or plant remnants, 2 type B and 2 weak toxins.

Total: 41 samples with 3 (5), or 7.3 (12.1) %, positive cultures.

Oklahoma.—Dr. L. L. Lewis, Oklahoma Agricultural and Mechanical College, Stillwater, forwarded, in April, 1921, 10 samples of soil (one-half to 5 inches depth, barnyard, poultry, sheep, cattle yard, hog lot) and 14 specimens of vegetables and plant remnants (decaying canna tops, decaying cauliflower, cinari, spinach tops, lettuce, tomatoes, sweet potatoes, radish roots, horse radish, onion roots, pea tops, decayed wheat straw, salsify roots and decayed cane hay). One culture prepared from decaying canna tops contained B. botulinus, type A. Horse radish, decayed cane straw and pea tops gave weak toxins.

Summary: 10 cultivated or manured soil, negative; 14 vegetables and plant remnants, 1 type B, 3 weak toxins.

Total: 24 samples with 1 (3), or 4.1 (12.5) %, positive cultures.

Oregon.—Four human and 2 fowl outbreaks; one at Hillsboro, due to home canned corn, one at Ontario, one at Klamath Falls and one at Grants Pass, all due to home canned string beans; 2 fowl outbreaks were caused by home canned string beans at Klamath Falls.

In August, 1920, E. Wagner collected and brought to the laboratory from Ashland, 3 samples of soil (barnyard, strawberry bed, pea garden); 2 specimens of fruits from the trees (cherries, bird pecked and sound) and 7 specimens of vegetables (beets, peas, and sugar peas, asparagus, bean vines, moldy vines, leaf mold, strawberries). The following results were obtained:

Sugar peas, leaf mold and asparagus tips contained B. botulinus, type A.

Soil from pea garden, barnyard and asparagus, beets, pea vines, moldy leaves (2), bean vine and cherries gave 9 weakly toxic cultures.

Dr. J. C. Geiger collected, in February, 1920, at Klamath Falls 2 samples of soil and 3 specimens of bean stalks and beans (chicken outbreak 26). The soil, one bean and the bean stalks contained B. botulinus, type A.

Dr. A. A. Soule of Klamath Falls forwarded, in September, 1921, 15 samples of soils. The cultures prepared with these specimens gave the following results:

Virgin soil (2), 2 B. botulinus, type A.

Garden soil (3), city pest house (1), 4 B. botulinus, type A.

Surface soil and swampy soil (2), weak toxins.

Garden soil (1), road soil (1), tule soil (1), tannery slough soil (1), surface dirt (1), spring yard (2), all negative.

Dr. H. J. Sears, Department of Bacteriology, University of Oregon Medical School, Portland, collected 2 samples of virgin, 3 of cultivated and 1 of nonfertilized soil (chicken yard) from the vicinity of Markham Hill. The cultures prepared with these specimens were nontoxic.

Summary: 4 virgin soils, 2 type A; 19 garden soils, barnyards, swamp soil, etc., 6 type A and 4 weak toxins; 12 fruits and vegetables, 5 type A and 7 weak toxins.

Total: 38 samples with 13 (24), or 34.2 (57.8) %, positive cultures.

Pennsylvania.—Two human outbreaks, one at Pittsburgh, due to home canned corn and one at Greensburg, caused by pickled olives packed in California.

Dr. George H. Hart, University of Pennsylvania Extension School, Philadelphia, forwarded, in April, 1921, 6 samples of soil (corral, corn field, poultry yard, pig pen, pasture, rye field) and 2 specimens of corn husks and beets. The following cultural results were obtained:

Soil from corn and rye field, 2 B. botulinus, type B.

Soil from field, pasture for 50 years, 1 B. botulinus, type A.

Soil from corral, 1 weak toxin.

In July, 1921, an additional 6 samples of moldy hay and corn from Montgomery and Chester counties, as well as moldy silage and hay were sent by Dr. Hart. Two specimens of silage were received from Pittsburgh, in May, 1921. The following cultures were toxic:

Sample of moldy hay, Chester County, B. botulinus, type B.

Sample of moldy corn fodder, B. botulinus, type A.

Summary: 6 manured or cultivated soils, 2 type B, 1 type A, 1 weak toxin; 10 feeds, silage, moldy hay, 1 type B, 1 type A.

Total: 16 samples with 5 (6), or 35.0 (37.5) %, positive cultures.

Rhode Island.—Dr. H. G. May, Agricultural Experiment Station of Rhode Island State College, Kingston, forwarded, in August, 1921, 6 samples of soil (rabbit pen, chicken yard, grain field, corn field, cattle pen and pasture) and 18 specimens of vegetables and feeds: silage, turnips (2), straw (2), dried beans, hay, beans (2), clover, beets (2), lettuce (2), spinach, peas, carrots and beet tops. The cultures prepared with these specimens proved nontoxic. One culture of spinach and soil of a corn field contained B. tetani.

Summary: 6 soils (cultivated or manured), all negative; 18 vegetables and feeds, all negative.

Total: 24 samples free from spores of B. botulinus.

South Carolina.—Dr. W. B. Aull, Associate Professor of Bacteriology, the Clemson Agricultural College, Clemson College, forwarded, in August, 1921, 7 samples of soil (hog pen, corn field, cow corral, grain field, poultry yard, clover field, cow pasture) and 15 specimens of vegetables and feeds (decayed leaves [2], beet tops, carrots [2], bean pods and stalks, beets, tops of kale, moldy hay [2], lettuce, pieces of corn stalks, tops of turnips [3]). The following cultures were toxic:

Beet and carrot tops, bean pods and stalks, 3 B. botulinus, type B.

Soil from grain field, 1 B. botulinus, type B.

Turnips, 1 weak toxin.

Summary: 7 soils manured or cultivated, 1 type B; 15 vegetables and feeds, 3 type B, 1 weak toxin.

Total: 22 samples with 4 (5), or 18.1 (22.7) %, positive cultures.

South Dakota.—Mr. C. D. Geidel, Director of the Minnesota Inspection Service, National Canners Association, St. Paul, forwarded, in January, 1921, 2 samples of soil and 2 specimens of corn husks procured from the Bigstone City Canning Company, Bigstone City. The cultures prepared with these specimens were nontoxic.

Dr. T. B. Taylor, Animal Health Laboratory, South Dakota State College, Brookings, forwarded, in April, 1921, 7 samples of soil (corn field [2], clover field, pig pen, cattle corral, pasture and poultry yard) and 6 specimens of roots (2), corn stalks, moldy hay and decaying vegetables (2). The cultures prepared with these were all nontoxic.

Summary: 9 soils cultivated or manured, negative; 18 vegetables and feeds, negative.

Total: 27 samples entirely free from spores of B. botulinus.

Tennessee.—One human outbreak in Memphis, due to ripe olives packed in California.

Dr. Maurice Mulvania, Department of Bacteriology, University of Tennessee, Knoxville, forwarded, in May, 1921, 7 samples of soil (corn field, cattle pasture, grain field, clover field, cattle pen, hog pen, poultry yard) and 13 specimens of vegetables and feeds (corn stalks, bean stover, bean stalk, pea vines, parsley, mustard, Irish potatoes, lettuce, beet stalk, young cabbage plant, straw [3]). The following cultures were toxic:

Irish potatoes, bean stalks, beet stalks, 3 B. botulinus, type A.

Soil from clover field, lettuce, mustard and decayed vegetation, 3 weak toxins.

Summary: 7 soils, cultivated or manured, 1 weak toxin; 13 vegetables and feeds, 3 type A, 2 weak toxins.

Total: 20 samples with 3 (6), or 15.0 (30.0) %, positive cultures.

Texas.—One human outbreak, due to sausages prepared in El Paso.

Mr. E. B. Reynolds, Division of Agronomy, Texas Agricultural Experiment Station, College Station, forwarded, in May, 1921, 7 samples of soil (cattle corral, pig pen, poultry yard, corn, grain, clover and pasture fields) and 14 specimens of vegetables and feeds (corn stalks, decaying vegetation [2], moldy hay [2], bean pods and stalks, peas, lettuce, beet tops, turnip greens [2], beets, radishes and parsley). The cultures were all nontoxic.

Summary: 7 soils, cultivated or manured, all negative; 14 vegetables and feeds, all negative.

Total: 21 samples entirely free from spores of B. botulinus.

Utah.—Dr. T. B. Beatty, State Health Commissioner, Utah State Board of Health, Salt Lake City, forwarded, in December, 1920, 2 samples of garden soil and 2 of unbroken sage brush, surface soil from the vicinity of Salt Lake City. One sample of garden soil contained B. botulinus, type A.

Vermont.—Professor B. F. Lutman, Department of Botany, University of Vermont, Burlington, collected and forwarded, in May, 1921, 9 samples of soil (pasture land, pig pen, cattle yard, corn field, chicken pen, forest humus, clover field, pig yard, barley field) and 13 specimens of vegetables, feeds, etc. (chaff [2], moldy silage, forest leaves, corn stalks, radishes, decaying weeds from compost heap, straw, bean pods, potatoes, beets, bean stalks and droppings from hen roost). The following cultures were toxic:

Decaying weeds from compost heap, 1 B. botulinus, type B.

Soil from pig yard, 1 B. botulinus, type B.

Summary: 9 soils cultivated or manured, type B; 13 vegetables, feeds and decaying vegetation, type B.

Total: 22 samples with 2, or 9.0%, positive cultures.

Washington.—Fourteen human and 23 fowl outbreaks; one human outbreak each at Seattle, due to home canned string beans and asparagus; one at McKenna, due to home canned spinach, 6 at Yakima, due to home canned corn, one at Yakima, due to home canned asparagus, one at Toppenish, due to home canned spinach, one at Yakima, due to commercially packed milk, one at Walla Walla, due to home canned asparagus and one at Monroe, due to commercially packed ripe olives. One chicken outbreak at Yelm, due to home canned string beans, 4 at Yakima, due to home canned corn and 2 due to home canned peas and 16 due to buried raw potatoes.

Mr. A. W. Hansen, Chief, Seattle Station, Bureau of Chemistry, U. S. Department of Agriculture, Seattle, forwarded, in January, 1921, 6 samples of virgin soil taken at Roosevelt Heights, just north of Seattle, 10 samples of commercial truck garden soil from South Park, sample of manure and 4 specimens of vegetables (potatoes, onions, carrots and turnips). The cultures prepared with these specimens were all nontoxic.

Dr. C. R. Fellers, Pacific Fisheries Investigation, National Canners Association, Seattle, collected and forwarded, in February, 1921, from the vicinity of the University grounds 11 samples of soil (garden, lumber yard, beet garden, subsoil, mud and silt, sewer opening, virgin, marsh soil, flower beds, drug plant garden [2]) and 1 sample of manure. The specimen of mud and silt produced a weakly toxic culture.

Dr. J. C. Geiger collected, in May, 1921, near Toppenish, Yakima Valley, 34 samples of garden soil (spinach garden [10], garden [18], irrigation ditches [4] and field soil [2]). The cultures gave the following results:

Spinach garden, 1 B. botulinus, type A, 2 type B, 1 weak toxin.

Vegetable gardens, 3 B. botulinus, type A.

Similar samples were collected at Tieton, Yakima Valley: 10 samples of ranch soil and 4 specimens of soil and potatoes from pits suspected of containing feed which caused avian botulism. The specimens developed toxic cultures, as follows:

Ranch soil, 3 B. botulinus, type A, 2 type B, 1 weak toxin.

Potato pits, 2 B. botulinus type A, 1 type B.

Additional samples were collected at random in the Yakima Valley: 6 samples of virgin soil, 6 of garden soil and 3 of pea garden soil. The following specimens developed toxic cultures:

Virgin soil, 5 B. botulinus, type A.

Garden soil, 1 B. botulinus, type A, 1 weak toxin.

Dr. J. C. Geiger collected, in July, 1921, near Yelm, 37 specimens of soil, manure, home canned peas and beans, etc. The following specimens furnished toxic cultures:

Eighteen soils, hen house (1), bean garden (6), cultivated (5), wheat field (3), pasture (1), garden (2), 17 B. botulinus, type A; 4 soil from irrigation ditches, 4 B. botulinus, type A; 3 virgin soils, 2 B. botulinus, type A, 1 weak toxin; 6 manures (hogs 3, cattle 3), 3 B. botulinus, type A, 1 weak toxin; 3 soil from house excavation, 3 B. botulinus, type A; 3 home canned peas (2), beans (1), 1 peas, 1 B. botulinus, type A.

In September, 1921, Dr. J. C. Geiger collected 20 samples of virgin soil along the eastern ledge of the Nisqually Glacier, Paradise Park, of the Mount Rainier National Park (elevation 5,000 to 6,000 feet); 7 cultures contained B. botulinus, type A, one type B and 5 gave weak toxins.

Summary: 36 virgin soils, 14 type A, 1 type B, 6 weak toxins; 41.6 or (58.3) %, positive; 98 garden, or irrigated soils, 32 type A, 4 type B, 4 weak toxins; 36.7 (40.8) % positive; 8 manure, 3 type A, 2 weak toxins; 8 vegetables and potatoes, 2 type A, 1 type B; 3 home canned peas or beans, 1 type A.

Total: 153 samples with 58 (70), or 37.8 (45.7) %, positive cultures.

West Virginia. — One human outbreak at Parkersburg due to pumpkin packed in Ohio.

Mr. Francis M. Morgan, Department of Soils, College of Agriculture and Agricultural Experiment Station, West Virginia University, Morgantown, collected and forwarded, in April, 1921, 8 samples of soils (poultry yard, cattle yard, horse pasture, clover, corn and wheat fields, pig pen, cold frames green house) and 12 specimens of moldy straw, corn stalks, decayed oat straw, cabbage heads, green peas, green beans, humus from compost heap, carrots, cucumber, radishes and manures from pig pen and horse stable.

One sample of soil from a field used for corn last year contained B. botulinus, type B.

Dr. J. C. Geiger collected 21 samples of virgin soil from the Alleghany Mountains in West Virginia.

One sample contained B. botulinus, type B, and one B. tetani.

Summary: 21 virgin soils, 1 type B; 8 manured and fertilized soils, 1 type B; 12 vegetables and manure, negative.

Total: 41 samples with 2, or 4.8%, positive cultures.

Wisconsin.—One human outbreak, due to commercially prepared sausages, at Milwaukee.

Mr. H. C. Kitchen, Director of Wisconsin Inspection Service, National Canners Association, Madison, forwarded, in December, 1920, 2 samples of soil and corn husk each from Columbus, 1 sample of pea vines and soil each, Cedar Grove, Thiensville and Bloomer. Ten cultures were prepared. One soil sample from Cedar Grove, Sheboygan County, produced a weakly toxic culture.

Dr. J. C. Geiger collected, in October, 1921, 19 samples of virgin soil with pine decay from wooded land, vicinity of Milwaukee, and 9 samples from a celery farm of the same region.

One sample of soil from wooded, virgin soil contained B. botulinus, type B, and one other produced a weak toxin.

One sample of soil collected from a celery farm gave a weak toxin, while three others contained B. tetani.

Summary: 14 cultivated and manured soils, 1 weak toxin; 17 virgin soil, 1 type B, 1 weak toxin; 5 vegetable remnants, negative.

Total: 36 samples with 1 (3), or 2.7 (8.3) %, positive cultures.

Wyoming.—One human outbreak at Cheyenne, due to home canned corn.

Dr. Cecil Elder, College of Agriculture and Mechanical Arts, University of Wyoming, Laramie, collected and forwarded, in April, 1921, 12 samples of soil [cattle corrals (6), pasture (2), hog pen (1), poultry yard, grain field and clover field] and 10 specimens of feeds and vegetables [moldy hay (5), moldy barley, pea pods and stalks, parsnips, carrots and potato].

The specimen of moldy barley contained B. botulinus, type A. The soil samples from the poultry yard, cattle corral, Ranch (1) and potatoes produced weak toxins.

In June, 1921, one of the writers (K. F. M.) collected 30 soil samples in the Yellowstone National Park. The cultures prepared gave the following results:

Forest soil near Old Faithful (2), 1 type A; upper Geyser Basin (9), rock-slide near Butterfly Geyser (1), all negative; road embankment near Castle Geyser (1), 1 type A; Forest soil near Saw Mill Geyser (2), 1 type A; Blacksand Basin (forest 2, swampy meadow 1), all negative.

Vicinity of Yellowstone Lake (8): Lake sand, near Hotel (1), negative; soil of damp meadow near Camp (1), negative; fresh soil cut near road side, Camp (1), 1 type A; soil on tree roots (2), all negative; soil from horse corral (1), 1 type A; soil from Stevenson Island (2), 2 type A.

Grand Canyon of the Yellowstone (8): Red volcanic rock near Lower Falls (1), street cut near Lookout Point (1), Terrace near Lower Falls (1), Meadow near hotel (1), sidewalk Lower Falls (1), all negative; damp soil under glacial bowlder near Canyon (1), 1 type B; summit of Mt. Washburn, damp soil (2), 2 type A.

Mammoth Hot Springs (5): Soil, squirrel hole on meadow (1), forest soil near Angel Trail (1), Devil's Kitchen (1), all negative; garden soil near hotel (1), 1 type A; soil near Boiling River (1), 1 type B.

Summary: 28 virgin soil (Yellowstone Park), 8 type A, 2 type B; 14 cultivated manured or garden soil, 2 type A, 2 weak toxins; 10 vegetables and feeds, 1 type A, 1 weak toxin.

Total: 52 samples with 13 (15), or 25.0 (38.8) %, positive cultures.

The statistical data presented in the preceding pages are summarized in table 1.

A brief analysis shows that 1,538 specimens, exclusive of California, have been studied; 375, or 24.3%, of the samples produced toxic cultures, although only 256, or 16.5%, could be identified by an antitoxin neutralization test. These figures are slightly changed by adding the findings reported for California, namely, 26.8%, or about one-quarter, of the field specimens collected in the United States contained spores.

which produced a B. botulinus-like poison in mass cultures. In 18.2% of the cultures, the presence of B. botulinus was definitely established by antitoxin tests.

TABLE 1

RESULTS OF EXPERIMENTS WITH SPECIMENS FROM VARIOUS STATES

State	Total Number of Specimens Examined	Total Number of Toxic Cultures	Total Number of Typed Cultures	Type A	Type B	Types A and B	Untyped	Percentage Total Toxic Cultures	Percentage Typed Cultures
Alabama	16	1	1	1	—	—	—	6.2	6.2
Arizona	1	—	—	—	—	—	—	0	0
Arkansas	41	6	1	—	1	—	5	14.6	2.4
(California)	(624)	(206)	(139)	(100)	(35)	(4)	(67)	33.0	22.2
Colorado	69	17	12	12	—	—	5	24.6	17.4
Connecticut	24	6	1	1	—	—	5	25.0	4.1
Delaware	18	6	4	—	4	—	2	33.3	22.2
Florida	45	9	8	1	7	—	1	20.0	17.7
Georgia	20	5	5	—	5	—	—	25.0	25.0
Idaho	33	7	4	3	1	—	3	21.2	12.1
Illinois	39	5	2	—	2	—	3	12.8	5.1
Indiana	28	8	5	—	5	—	3	28.5	17.8
Iowa	34	3	1	1	—	—	2	8.6	2.9
Kansas	30	2	2	2	—	—	—	6.6	6.6
Kentucky	17	12	12	1	11	—	—	70.5	(70.5)
Louisiana	48	6	2	2	—	—	4	12.5	4.1
Maine	41	10	10	8	2	—	—	24.3	24.3
Maryland	30	17	15	1	12	2	2	56.6	50.0
Massachusetts	17	3	1	—	1	—	2	17.6	5.8
Michigan	120	10	5	1	4	—	5	8.3	4.1
Minnesota	46	5	2	2	—	—	3	10.8	4.3
Mississippi	19	6	3	—	3	—	3	31.5	15.7
Missouri	34	15	12	1	11	—	3	44.1	35.2
Montana	66	27	16	12	3	1	11	40.8	24.2
Nebraska	23	6	2	2	—	—	4	26.0	8.6
Nevada	25	11	9	9	—	—	2	44.0	36.0
New Hampshire	14	2	1	—	1	—	1	14.2	7.1
New Jersey	36	9	7	1	6	—	2	40.0	19.4
New Mexico	8	—	—	—	—	—	—	0	0
New York	31	8	6	6	—	—	2	25.8	19.3
North Carolina	20	6	—	—	—	—	6	30.0	0
North Dakota	14	3	1	1	—	—	2	21.4	7.1
Ohio	41	5	3	—	3	—	2	12.1	7.3
Oklahoma	24	4	1	—	1	—	3	16.6	4.1
Oregon	38	24	13	13	—	—	11	63.1	34.2
Pennsylvania	16	6	5	2	3	—	1	37.5	35.0
Rhode Island	24	—	—	—	—	—	—	0	0
South Carolina	22	5	4	—	4	—	1	22.7	18.1
South Dakota	17	—	—	—	—	—	—	0	0
Tennessee	20	6	3	3	—	—	3	30.0	15.0
Texas	21	—	—	—	—	—	—	0	0
Utah	4	1	1	1	—	—	—	25.0	25.0
Vermont	22	2	2	—	2	—	—	9.0	9.0
Washington	153	70	58	52	6	—	12	39.2	37.8
West Virginia	41	2	2	—	2	—	—	4.8	4.8
Wisconsin	36	3	1	—	1	—	2	8.3	2.7
Wyoming	52	16	13	11	2	—	3	30.7	25.0
Total (exclusive of California)	1,538	375	256	150	103	3	119	24.3	16.5
Total (inclusive of California)	2,162	581	395	250	138	7	186	26.8	18.2

One hundred and fifty, or 58.5%, of the typed cultures contained B. botulinus, type A; 103, or 40.3%, type B, and 3, or 1.2%, mixture of type A and B. The predominance of type A is probably due to the findings made in the State of Washington, and is of little significance. In fact, it is not the relative numerical preponderance of one type over the other, but the geographical distribution of the various types which deserves special consideration in subsequent paragraphs. Attention is called to the negative findings made on the samples procured from New Mexico, South Dakota and Texas. It is quite likely that these results indicate an uneven distribution of the spores of B. botulinus, but it would be unwise to conclude from the limited data that the organism is not present in the soil of these states. The specimens collected in Arkansas, Connecticut, Illinois, Indiana, Louisiana, Michigan, Minnesota, Mississippi, North Carolina, North Dakota, Oklahoma, South Carolina, Tennessee and Wisconsin furnished a relatively large percentage of cultures which were weakly toxic and could not be identified by antitoxin tests. It was also noted that the typed cultures prepared with the samples obtained from these states contained only 1 to 5 minimum lethal subcutaneous guinea-pig doses per 2 c c. Previous studies have shown that specimens collected in California, Oregon, Washington, Montana, and other states, yield, as 25 gm. samples during 10 days' incubation in peptic digest broth, toxic cultures which are fatal to guinea-pigs in dilutions of 1 : 1,000 to 1 : 10,000. Not infrequently, B. botulinus has been isolated in a pure state from these cultures, while innumerable attempts made with the specimens from the first named states, in which no strongly toxic cultures were found, have regularly been unsuccessful. Laboratory tests reported in a former paper indicate that a few spores added to soils produce, as a rule, weakly or nontoxic cultures. It is naturally possible that a number of weakly toxic cultures are the result of the recently described anaerobe isolated by Bengtson from the larvae of Lucilia Caesar, although repeated transplants and cultures of new samples of soil which originally gave weakly toxic cultures furnished in a few instances highly potent enrichments containing the toxin of B. botulinus. One is therefore forced to conclude that the field specimens collected in the 14 states mentioned harbored relatively few spores of B. botulinus. The practical significance of this observation will be considered in greater detail in the following.

The data presented in table 1 suggest a number of interesting points, which will be treated under the following headings:

(1) The relative distribution of B. botulinus in different states of the United States.

(2) The distribution of the two types of B. botulinus.

(3) The telluric distribution of the spores.

(4) The relation of the distribution of B. botulinus in the soil and its products to outbreaks of human and animal botulism.

(1) *The Relative Distribution of B. botulinus in Different States of the United States.*—With the exception of Virginia, every state of the Union supplied from 1 to 624 samples of soil and its products. Nevertheless, the survey cannot be considered in any way complete or exhaustive. Such a study is obviously very difficult and would consume an enormous financial outlay and several years of continuous work. A scrutiny of table 1 indicates that a number of states provided a small number of specimens which necessarily represent confined areas of the state. These facts should be fully appreciated and the deductions which will be made from the 1,538 cultural findings should be considered tentative, although they are in many ways suggestive.

For the sake of clearness and brevity, the figures dealing with the percentage of toxic and properly identified cultures are presented on chart 1. Four contrasting shades representing the percentages of 1 to 10 (1), 10 to 20 (2), 20 to 30 (3), and 30 to 70 (4), have been chosen for graphic illustration of the distribution in the different states. A rapid glance at chart 1 reveals three interesting facts, namely:

(1) The spores of B. botulinus are relatively rare in the samples of soil, etc., collected from the Middle States, the Great Plains States and those bordering the Great Lakes and the Mississippi River with its tributaries, the Missouri, Arkansas and Red, Des Moines and Illinois Rivers.

(2) The Atlantic States supplied specimens which produced frequently from 10 to 30% toxic cultures.

(3) The soil of the Pacific Coast and Rocky Mountain States was heavily infected with B. botulinus.

One state of the 14, bordering on the Mississippi River, namely, South Dakota, supplied nontoxic cultures, while Kentucky furnished 70.5%, Mississippi and Tennessee 15% and Missouri 35.2% toxic cultures. In Kentucky and in Missouri, collections were made in the vicinity of experiment stations. As far as Kentucky is concerned, it is known that for a number of years work on animal botulismus has been carried out at the experiment station, and it is not unlikely that the percentage of positive specimens can be attributed to contamination of the premises with spores. The samples from Tennessee originated in the neighborhood of Knoxville, or in close proximity to the Appalachian Mountains. Excluding these 4 states from consideration, it is clear that the soil of the Mississippi Basin contains the spores of B. botulinus in exceptional instances only. In fact, 9 states which are represented in this study by 311 specimens or one-fifth of all the samples tested in the United States, exclusive of California, gave less than 4.5% positive cultures. Sixty-four per cent. of these toxic cultures were weakly toxic and were probably the result of a very few heat resistant, viable spores.

The states of Michigan, Indiana, Ohio, possibly Pennsylvania and West Virginia, form a part of a region which has a low B. botulinus spore index. This statement is particularly well supported by the findings made on 120 specimens collected in Michigan; 62 soil samples derived from cultivated fields gave 3, or 4.8%, toxic cultures, while 10 virgin samples proved on repeated tests to be free from B. botulinus. Ohio and West Virginia gave 7.1 and 4.8% toxic cultures. One locality in Indiana supplied 17.8% positive samples, which contained the toxicogenic anaerobe, but judging from the number of additional negative specimens, this state can well be included in the group mentioned. It is regrettable that most of the material on which the data for Pennsylvania are based were collected in the eastern section of the state, and no information relative to the frequency of B. botulinus in the western part along the Alleghany and Ohio Rivers is available. Notwithstanding this fact, it appears that the valley of the Ohio River shares the same low percentage of B. botulinus in the soil as the Mississippi Basin and the lower portion of its tributary, the Missouri. The

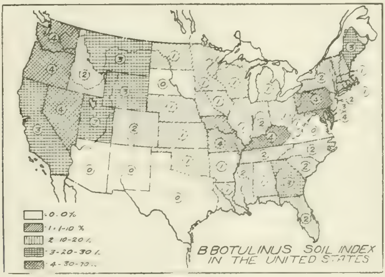

Chart 1

15 states which furnished less than 10% positive cultures belong topographically to the Great Plains. The average altitudes of these states from which samples have been collected are between 1,000 to 2,000 ft. It is impossible to connect the limited data thus far collected with any geological formation. Most of the soil samples belong to the class of residual rock. Future studies must furnish the solution of the many questions which these observations suggest, but it is believed that the low B. botulinus index in the Great Plains and the Mississippi Valley is, in the light of the observations made in California, mainly governed by the absence of heavily infected virgin mountain areas.

Six of the 15 North and South Atlantic States supplied soil and vegetables which contained B. botulinus in from 20 to 50% of the cultures, while those derived from 4 states, namely, Vermont, New Hampshire, Massachusetts and Connecticut, were positive in less than 10%, usually 5%, of the tests. These two extremes are balanced by the findings in the states of Florida, North and South Carolina, New Jersey and New York, which furnished between 10 to 20% typical cultures. As a whole, 15 Atlantic States, represented by 421 specimens, gave 96, or 22.8%, toxic and 71, or 16.9%, typable cultures, which were definitely proved to contain B. botulinus. This organism is, therefore, somewhat more frequently encountered in the Atlantic States than in those of the Great Plains and the Mississippi Valley. Pennsylvania and Maryland are exceptions, but it is not unlikely that the high percentage of positive cultures is the result of chance sampling or is dependent on the same soil stratum in both states, namely, a part of the Blue Ridge Mountain soil which supplied the samples. The relatively frequent findings of B. botulinus spores in the draining areas of these mountains, namely, New Jersey, Delaware and Maryland, lends considerable support to the last mentioned view and suggests the Blue Ridge Mountains as a breeding place for the neighboring valleys and river beds.

The number of examinations made on material supplied from the 4 North Atlantic States which gave less than 10% positive cultures is too small to justify any definite conclusions. The states are surrounded on both sides by soil stratums which harbor B. botulinus spores in a fairly large percentage of specimens. Attention is called in this connection to the existence of the organism in virgin forest soil in Main and New York. It is therefore not unlikely that extended sampling in Vermont, New Hampshire, Massachusetts and Connecticut may radically change the aspect of the data thus far collected.

The soil and vegetable specimens obtained from the South Atlantic States, Georgia, North and South Carolina, originated in the interior, while those of Florida represent coast, as well as inland, collections. Georgia and South Carolina yielded 25 and 18.1% positive cultures; soil strata of the foothills of the Blue Ridge Mountains supplied the samples. In Florida, the differences between the coast and the interior are clearly shown by the absence of B. botulinus in the samples collected around Miami and the fairly high percentage of infected material procured from the neighborhood of Gainesville. This interesting comparison indicates that virgin soil on the Atlantic Coast may be free from the spores of B. botulinus and, furthermore, emphasizes the conclusion previously drawn that the findings made on one topographical section do not necessarily apply to the state as a whole.

The results reported for the Middle and Atlantic States are in striking contrast to those revealed by the specimens procured from the Western States. Ten states, exclusive of California, supplied 449 samples, with 126, or 28%, toxic and properly identified cultures. Topographically these states form a part of the Cordilleran system, and, with the exception of Colorado and Idaho, furnish a B. botulinus soil index of 20%, while in 3 states, Oregon, Nevada and Washington, it is more than 30%. In the last named state, every section was found to contain this anaerobe, and the detailed findings made in the Yakima Valley deserve brief consideration. The percentage figures shown in chart 2 suggest that B. botulinus is, as in California, an inhabitant of the virgin soil. It may be mere coincidence due to methods of sampling, but the data indicate that from the mountain ranges toward the valleys, following the descending course of the rivers, there is a progressive reduction in the infection index of the soil. For example, virgin mountain soil contained B. botulinus spores in 83% of instances, while earth collected in the adjacent Tieton and Yakima Valley sections gave 57.1 and 55.5% positive cultures. On the other hand, the Toppenish area, which is farther removed from the source of the Yakima River and its tributaries, supplied comparatively few infected soil specimens (11.7%). The soil is equally polluted in the vicinity of Yelm and the virgin soil along the eastern ledge of the Nisqually Glacier on Mount Rainier.

The demonstration and isolation of B. botulinus from virgin sage brush soil and uninhabited sections of Nevada forcibly support the conclusion that this organism is universally distributed in the Western States. Additional evidence was also secured by carefully sampling virgin districts in 3 National Parks: Yellowstone, Glacier and Mount Rainier, as well as the region surrounding Pike's Peak. Twenty-six to 40% of the soil samples collected in these regions yielded positive cultures and frequent isolations of the anaerobe. B. botulinus has been located at altitudes around 11,000 ft. in soil layers of glacial origin and on an isolated island of the Yellowstone Lake and has, by repeated tests, been demonstrated in pure cultures. This evidence strongly refutes the conception of an intestinal habitat of the organism. Unfortunately little or no attention was paid to the geological structures which furnished the positive virgin soil specimens, and this survey does not permit an answer to the question: Can B. botulinus breed in these regions, and what are its biological functions? Work to solve these mysteries is in progress and will be reported when available.

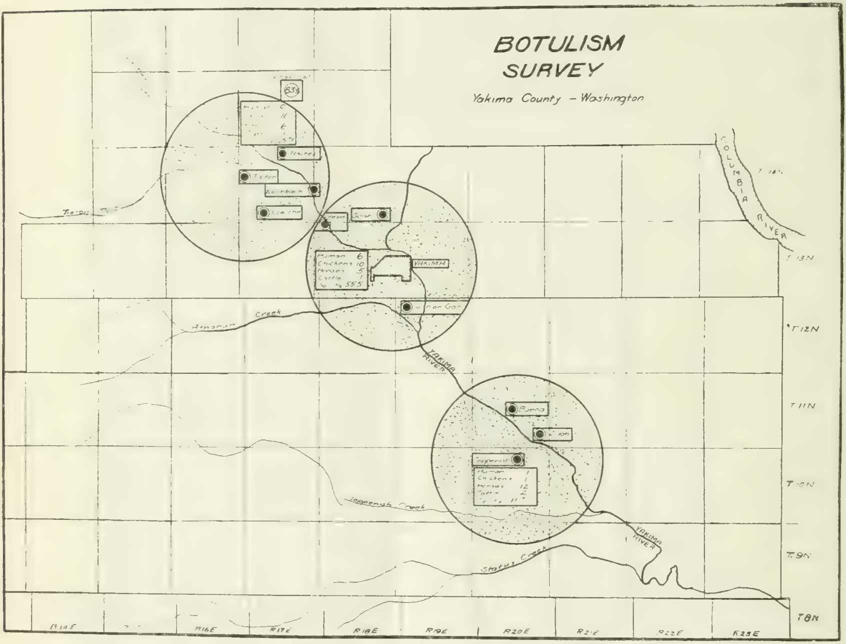

Chart 2

Based on this survey, it must be concluded that B. botulinus is a common soil anaerobe of the Western States or the Cordilleran system; it is less frequently encountered in the Atlantic States and relatively rare in the Middle States, Great Plains and the Mississippi Valley.

(2) *The Distribution of the Two Types of B. botulinus.*—Through the early studies of Leuchs [2] and the recent work of Burke,[3] it is known that the antitoxin prepared against types A and B toxins is specific for the homologous toxin and will not protect against the heterologous one. This fact has been used to identify the toxin generated in the cultures prepared with the field specimens. A total of 256 cultures has been typed or identified by the isolation of B. botulinus in pure culture. It was recognized that certain states furnished only one type of B. botulinus, namely, type A or B, while some gave, for example, predominantly type A, with one or two cultures yielding type B toxin, and vice versa. By grouping these data according to states and by using 4 contrasting shades, chart 3 has been obtained.

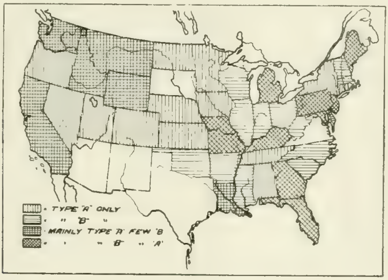

Chart 3

It is quite evident that the distribution of the 2 serologic types of B. botulinus divides the United States roughly into 2 distinct areas: The Western States, inclusive of the Great Plains, and the Mississippi Valley together with the Atlantic States. In a general way, the soil area spreading from the 95 degrees longitude westward to the 125 degrees longitude harbors mainly type A, while that extending from 95 degrees longitude eastward to the 65 degrees longitude supplies B. botulinus type B. The Mississippi Valley, with the valleys of its tributaries, the Ohio, the Red and a part of the Missouri, as well as the Great Lakes region, is characterized by a striking predominance of type B. Similarly prevalent is this type in the Atlantic States of Maryland, Delaware, New Jersey, Georgia, South Carolina, etc., while the scattered findings of type A, in Maine, New York and Pennsylvania indicate the probable existence of seed beds in the form of virgin forests and mountains. Alabama, Connecticut and Tennessee supplied only type A toxins, but it is not unlikely that more extensive sampling would also place these 2 states in the type B. area.

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[2] Ztschr. f. Hyg. u. Infektionskr., 1910, 65, p. 55.

[3] Jour. Bacteriol., 1919, 4, p. 555.

For several years, it was known that type A was prevalent in the Western States, and the data illustrated by chart 3 fully confirm the statement made in a former paper that this type is primarily an inhabitant of the virgin soil from which it may be scattered by the streams and rivers to distant regions, even invading the Missouri and Mississippi Valleys (see Missouri and Louisiana). In Colorado, Oregon, Nevada, Utah, North Dakota, Nebraska, etc., type A is the only type encountered, while in Washington, Idaho, Montana and Wyoming type B is occasionally found; in every instance, the specimens infected with the spores of the latter type were either soil or vegetables from gardens or forest or moraine soil rich in decayed plant material. One sample of moldy alfalfa hay from Bozeman, Mont., contained a mixture of type A and B. The facts so revealed agree perfectly with the data presented in a former paper, namely: Type B is probably an adaptation mutant to the physical and chemical influences encountered by type A in tilled and cultivated soil. Whether the same explanation applies to the conditions in the Middle and Atlantic States cannot be stated with certainty. It may be mere coincidence, but by comparing chart 3 with a recent map showing the density of population in the United States according to the 1920 census, it will be noted that the type B area is nearly covered by that holding a population of from 18 to 400 persons per square mile. In other words, the soils which are subjected to intensive cultivation and fertilization contain, as a rule, B. botulinus, type B. A similar condition apparently exists in Canada and on the European continent.

Further comparison of chart 3 with chart 1 indicates that the specimens derived from states which yielded only, or at least predominantly, type B, produced also a low percentage of toxic cultures. When type B is isolated, the number of spores in the soil and its products is relatively small. This interesting condition was first noted in California and has since then been confirmed by the studies made in other states of the

Union. These facts have an encouraging aspect from the standpoint of botulism prevention, namely, intensive agriculture may in generations suppress B. botulinus as a soil anaerobe and consequently eliminate the danger of spore contamination on vegetables, fruits and feeds, provided the rivers and streams draining heavily infected areas of type A cease to reinfect these districts. Extensive irrigation as practiced in California and Colorado has been and is probably to a great extent responsible for the pollution of the cultivated fields and orchards with highly heat resistant spores of B. botulinus, by nature only present in the mountain soil. The significance of type B in its relation to human botulism is discussed in a subsequent paragraph.

(3) *The Telluric Distribution of B. botulinus.*—In chart 4, the cultural results of the 1,538 field specimens are arranged in graphic form according to their telluric origin, namely, virgin, cultivated, garden and pasture soil, dirt from animal corrals and vegetables and feeds. This presentation emphasizes the predominance of B. botulinus in virgin and pasture soil and the relatively low percentage of positive cultures in soil and manure collected from animal corrals, pig pens and chicken houses. As a whole, the data correspond closely to those already mentioned for California. Earth contaminated with manure or animal excreta rarely carries the spores of B. botulinus. In fact, fertilization cannot be considered a factor contributing to the pollution of the soil. Moreover, these columns are responsible for the suggestions made in the preceding paragraphs, namely, cultivation and cropping of the soil reduces or dilutes the number of spores to such a degree that the methods employed for detection fail to demonstrate their presence.

This view is also supported by the examination of soil products. Soil stratums furnishing nontoxic or weakly toxic cultures frequently supplied vegetables or feeds, which contained B. botulinus, type B. It is possible that the concomitant anaerobes or aerobes of the cultivated or manured soil inhibit the growth or the toxin production of the few spores of B. botulinus present in the usual amounts chosen for these tests. As a rule, the vegetable samples examined have been less heavily polluted with various antagonistic soil bacteria and demonstration of the toxicogenic anaerobe has been more readily accomplished than with soil specimens. In any case, 1 gm. of cultivated or manured garden soil contains quantitatively fewer spores than the same amount of virgin soil.

The cultural findings made on vegetables and feeds reflect the flora of the soil and may indicate the potential danger from B. botulinus in a community. In this connection, it should, however, be emphasized that the mere demonstration of the organism in feeds does not signify that this material has in the past or may in the future cause animal

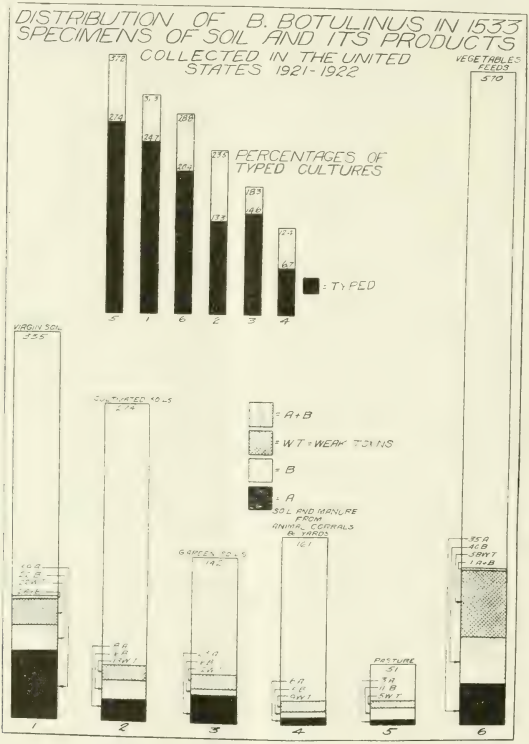

Chart 4

botulism. A number of workers dealing with certain animal diseases in the Middle Western States have repeatedly failed to appreciate these facts, and they have invariably made the diagnosis of botulism provided they were able to isolate B. botulinus from the suspected feed or the spleens and livers of dead horses, swine and sheep.

In table 2, the various vegetables, fruits and feeds which have been examined are tabulated and the percentages of positive cultures have been calculated for each important plant material. There is no doubt that every soil product may sometimes carry the spores of B. botulinus, but in the light of the epidemiology of botulism in this country, it is significant that string beans and leaves furnished the highest percentage of toxic and identified cultures. In this connection, it should be recalled that 45 instances are on record in which home or commercially packed string beans caused outbreaks of human (20) and avian (25 botulism. The factors responsible for the frequent contamination of string bean pods or vines deserve more careful experimental investigation, not

TABLE 2

PERCENTAGE OF POSITIVE CULTURES IN VARIOUS VEGETABLES, FRUITS AND FEEDS

Vegetables and Feeds	Total Number Examined	Number of Positive Cultures	Percent of Positive Cultures
Cornhusks, leaves and stalks	80	6	7.5
Peapods, stalks and leaves	51	3	5.4
Moldy hay	44	7	**15.9**
String beans, pods and stalks	44	14	**32.7**
Beets, roots and tops	37	6	16.2
Decayed vegetation	29	6	**20.6**
Tomato plant and roots	24	2	8.3
Lettuce	21	3	14.2
Carrots	18	2	11.1
Turnips	18	2	11.1
Cabbage	16	3	18.7
Ensilage	15	3	**20.0**
Radishes	15	1	6.6
Decayed straw	13	1	7.6
Straw	10	0	....
Bean roots	10	1	10.0
Spinach	10	0	....
Lima beans	9	1	11.1
Cucumber root	8	0	....
Pumpkin vines	8	0	....
Sweet potatoes	8	1	12.5
Swiss chard	8	0	....
Potatoes	8	2	25.0
Onions	8	1	12.5
Forage	6	2	33.3
Alfalfa	4	1	25.0
Parsnips	4	0	....
Asparagus	4	1	25.0
Oats	4	1	25.0
Parsley	4	0	....
Soya beans	4	1	25.0
Cherries	3	0	....
Dandelions	2	1	....
Hominy	2	0	....
Beet pulp	2	0	....
Mango tops	2	0	....
Red pepper	2	0	....
Mustard	2	0	....
Daffodils, cornfodder, wheat head, squash, crabapple, rutabaga, moldy barley, canna tops, cauliflower, curari roots, clover, strawberries (one each)	13	3	....
Total 50 soil products	570	75	

only from a biologic but also from a preventive standpoint. Plant material undergoing decay and fermentation, as for example vegetables, moldy hay and ensilage, supplied a high percentage of toxic cultures and isolations of B. botulinus. According to the reports accompanying the shipments of these samples, it was known that some of the moldy hay and ensilage containing B. botulinus had been consumed by horses and cattle with impunity. A few toxicologic examinations conducted in this laboratory failed to demonstrate the presence of B. botulinus toxins in these samples. These and similar observations cast serious doubt on the majority of claims which in recent years associate any mysterious animal disease with botulism. In fact, it is evident from the data presented in table 2 that B. botulinus is frequently found in moldy hay and ensilage but, so long as these feeds are free from toxin, they may be ingested by herbivorous animals without causing botulism.

From a biologic standpoint, it is by no means surprising to find B. botulinus frequently associated with decaying plant material. The organism, being a spore-bearer, rarely meets in nature the conditions suitable for its growth, and its continued existence would be impossible without some resting stage. In soil, conditions favorable to the growth probably occur when decaying vegetation furnishes the necessary food material. These conditions probably develop just often enough so that the spores do not diminish in number. This behavior deserves further study, but this possibility should be fully appreciated in canneries and utmost care exercised in the disposal of waste products. It is not unlikely that the most resistant spores of B. botulinus are not introduced into the packing plant on the fresh and sound vegetables and fruits, but are made available under unsanitary conditions of waste disposal. The principles which apply to the suppression of heat resistant thermophiles can unreservedly be used in the control of B. botulinus spores.

A total of 50 specimens of beans, cucumber and tomato roots, not tabulated, revealed only one positive culture, while the vines and leaves of the same plants from the same source furnished 10 or 20% positive cultures. These and similar tests should be made on a larger series of samples, but they confirm in a general way the previously made observations that B. botulinus occurs more often in the surface layers of the soil and consequently can cause dust borne contaminations of sound fruits on the trees. This condition has repeatedly been noted in the olive orchards of California.

TABLE 3

Number of Outbreaks of Botulism Compared with Percentage Figures of Soil and Vegetable Specimens Containing Spores of B. botulinus

State	Botulism Outbreaks						Percent of Specimens Containing B. botulinus Spores			
	Human				Fowl: Locally Grown, Home Packed Food Products	Horse and Mules: Locally Grown Feed, etc.				
	Total	Locally Grown, Commercially Packed	Locally Grown, Home Packed or Preserved	Unknown			Typed	Type A	Type B	Type A and B
Arizona	1	(1 Ohio)	..	..	..	..	....	.....	.....	.....
California	47	11 (1 Ohio)	29	6	53	?	22.2	78.4	22.1	3.1
Colorado	3	1 (?)	1	..	3	(1)	17.4	100.0	.....	....
Dist. Columbia	..	..	..	..	1	..	....	.....	.....	.....
Florida	1	..	1	..	..	..	17.7	12.5	87.5	.....
Idaho	3	..	3	..	..	..	12.1	75.0	25.0	.....
Illinois	1 (?)	1 (?)	..	..	1	1	5.1	.....	100.0	.....
Indiana	3	.. (2 California)	1	..	..	..	17.8	.....	100.0	.....
Iowa	1(?)	..	1(?)	..	..	..	2.9	100.0	.....	.....
Kansas	..	1 (?) (Colorado)	..	..	..	..	6.6	100.0	.....	.....
Kentucky	..	..	..	..	..	1	70.5	.....	100.0	.....
Maine	1	..	1	..	..	..	24.3	80.0	20.0	.....
Massachusetts	3	..	2	1	..	..	5.8	.....	100.0	.....
Michigan	3	0 (3 California)	..	..	..	..	4.1	20.0	80.0	.....
Montana	1	0 (1 California)	..	..	..	..	24.2	75.0	18.7	6.3
Nevada	..	..	..	..	5	..	36.0	100.0	.....	.....
New Jersey	1	..	1	..	..	..	19.4	14.3	85.7	.....
New York	5	0	3	1	..	..	19.3	100.0	.....	.....
Ohio	2	3 (California, Arizona, W. Virgina) (2 California)	..	..	..	..	7.3	.....	100.0	.....
Oregon	4	0	4	..	2	..	34.2	100.0	.....	.....
Pennsylvania	2	0 (1 California)	1	..	..	..	35.0	40.0	60.0	.....
Tennessee	1	0 (1 California)	..	..	..	..	15.0	100.0	.....	.....
Texas	1	1	..	..	..	..	0	0	.....	....
Washington	14	1	12	1	23	..	37.8	89.5	11.4	.....
West Virginia	1	0 (1 Ohio)	..	..	..	..	4.5	.....	100.0	.....
Wisconsin	1	1	..	..	..	..	8.3	.....	100.0	.....
Wyoming	1	..	1	..	..	..	25.0	84.7	15.3	.....
Total 27 states	102	32	61	9	88	3				

(4) *Relation of Distribution of B. botulinus in the Soil and Its Products to Outbreaks of Human and Animal Botulism.*—In table 3, the number of botulism outbreaks thus far recognized in the different states is compared with the percentage figures of soil and vegetable specimens found to contain the spores of B. botulinus.

It is not surprising to note that when the percentage figures of positive cultures exceed 20 or 30%, and particularly when type A predominates, human and animal botulism is not infrequent. California, Colorado, Idaho, Maine, Nevada, New York, Oregon, Washington and Wyoming are excellent examples of these conditions. The states furnishing B. botulinus, type B either report no outbreaks, or botulism is caused by food products which have not been preserved by heat (sausages, hams, etc.). Comparative studies have shown that the spores of the majority of B. botulinus, type B strains are, as a rule, less heat resistant than those of type A (average of 23 strains type B, at 105 C. = 37 minutes and of 33 strains type A, at 105 C. = 62 minutes). It is, therefore, not unlikely that the absence of botulism in the states, which primarily harbor type B is due to the relative scarcity and possibly the low heat resistance of the prevailing spores of B. botulinus. This explanation is based on the strains thus far available, but it should be stated with utmost emphasis that spores of type B have been grown in this laboratory which have withstood a temperature of 105 C. for at least 65 minutes (strains 13, 41, 77 and 63) or the average resistance of type A strains.

No assurance can be given that such spores may not occasionally enter the food product to be preserved, and it would be most unwise to disregard in a Middle Western or Eastern state vigilance and rigid procedures of sterilization merely on account of the conditions indicated by this survey. From a practical standpoint, the spores of B. botulinus are ubiquitous; they may be found anywhere and any time, although certain locality variations deserve the attention of the biologist.

Outbreaks of fowl botulism are usually due to preserved vegetable products, and the same factors which lead to human cases are responsible for these fatalities. In fact, an approximate estimate of spoilage due to B. botulinus can be made from the various outbreaks of botulism among barnyard fowls, which developed symptoms of the disease or died following the consumption of poisonous, spoiled food. It is to be regretted, therefore, that reliable epidemiologic data relative to the number of outbreaks of botulism among fowls in states other than California and Washington are not available. On observation, in which spoiled corn containing B. botulinus type B was the cause, has been reported from Washington, D. C. In another, which occurred in New Jersey, the type of the organism was not determined; while B. botulinus type A was found incidentally in a sample of chicken manure collected at East Lansing. By analogy with human outbreaks, it must be suspected that localities yielding a high percentage of B. botulinus in soil will furnish outbreaks of avian botulism. This statement deserves additional investigation and is made with reserve, as dependable data are not available. The same must be said with regard to the so-called outbreaks of equine, bovine and porcine botulism. In California, where every opprotunity has been selected to verify the clinical diagnosis of equine botulism by careful laboratory tests, it has been impossible to find even one instance in which the suspected moldy hay, corn or straw contained the toxin of B. botulinus. The bacteriologic demonstration of B. botulinus in the tissues of horses and of cattle cannot be used as a criterion or as proof that certain forms of "forage poisoning" are actually botulism. Laboratory findings also indicate that this anaerobe may sometimes be demonstrated in the organs of animals which die of other diseases than botulism. Furthermore, states with a relatively low B. botulinus soil index report extensive outbreaks of "forage poisoning," and vice versa. In the light of these conditions, it is obviously impossible to draw deductions which can demonstrate any relationship between spore prevalence and equine or bovine botulism.

CONCLUSIONS

A general survey, during which 1,538 soil, vegetable, feed and manure specimens of every state of the United States, except Virginia, have been studied for the presence of the spores of B. botulinus, reveals the following facts:

1. B. botulinus is a common soil anaerobe of the Western States of the Cordilleran system. It is less frequently encountered in the Atlantic States and is relatively rare in the Middle States, the Great Plains and the Mississippi Valley.

2. The soil of the Western States, inclusive of the Great Plains, yield, mainly, B. botulinus, type A, while the Mississippi Valley and Great Lakes region is characterized by a striking predominance of type B. Similarly prevalent is this latter type in the Atlantic States of Maryland, Delaware, New Jersey, Georgia and South Carolina, while scattered findings of type A in Maine, New York and Pennsyl-

vania indicate the existence of breeding places in virgin forests and mountains. Soils which are subjected to intensive cultivation and fertilization contain, as a rule, B. botulinus, type B.

3. B. botulinus spores are far more prevalent in virgin and pasture soils than in dirt, soil or manure collected from animal corrals, pig pens, etc. Vegetables, fruits and feeds are frequently contaminated with the spores of B. botulinus. String bean pods and leaves, moldy hay, ensilage and decayed vegetation may yield a relatively high percentage of positive cultures.

4. Human and animal botulism is not infrequent in those states in which B. botulinus, type A, predominates, or in which the percentage figures of positive cultures exceeds 20 to 30%. From a practical standpoint, however, B. botulinus is ubiquitous, and this survey gives no assurance that heat resistant spores cannot be found anywhere and at any time.

5. The theory which claims that all the pathogenic anaerobes are regular inhabitants of the intestinal canal of animals deserves renewed investigation in the light of this survey on B. botulinus.

THE DISTRIBUTION OF THE SPORES OF B. BOTULINUS IN THE TERRITORY OF ALASKA AND THE DOMINION OF CANADA. V *

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In order to supplement the findings reported in the preceding papers, a limited number of soil specimens obtained from Alaska and the Dominion of Canada have been examined. This bacterio-geographic study serves to answer two questions: 1. Does B. botulinus occur in the soil of Alaska, and is the salmon canning industry endangered by this organism? 2. Can B. botulinus be demonstrated in the virgin forest and mountain soil of Canada?

No outbreaks of botulism due to commercially canned salmon have thus far been reported, probably on account of the fact that B. botulinus produces rapid and marked spoilage, which leads to an elimination of the abnormal tins before they reach the consumer. From a biologic and topographical standpoint, it was of interest to search for B. botulinus along the Alaskan river banks and Coast stations which house the various salmon canneries. Previous studies had shown that the rivers of the Pacific Coast slope serve as distributors of the spores from the mountains, which are the natural breeding places, so that the finding of B. botulinus, type A, in two sound clams removed from San Francisco Bay was not surprising. It was originally contemplated to study soil specimens collected at various sections of the Cordilleran system, extending along the Pacific Coast from the state of Washington to the Aleutian Islands. Unfortunately, for financial reasons, such a systematic survey was impossible, and the few samples which were procured from Alaska can only serve as guides for future investigations.

With the exception of one outbreak, reported by Glancy[1] and supposedly caused by government inspected meat or canned beets near Dawson City, Yukon, no human or animal botulism cases have been reported from Canadian territory. Samples of soil have been collected from various regions of the Dominion, and the results of the examinations form the basis for this communication.

Received for publication, July 26, 1922.

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* This study was aided by grants from the National Canners Association, the Canners League of California and the California Olive Association.

[1] Canadian Med. Assn. Jour., 1920, 10, p. 1027; Report 82 in the Epidemiology of Botulism, Bullet. U. S. Public Health Service, in press.

EXPERIMENTAL DATA

The methods outlined in the preceding papers have been followed in the collection and examination of the specimens. The results are herewith briefly reported.

Alaska. — Dr. E. D. Clark, Director of Pacific Fisheries Investigations, National Canners Association, Seattle, Wash., forwarded, in September, 1921, 7 samples of soil, which had been collected during the summer, at the following stations in Alaska: Alitak, Chignik River, Larsens Bay, Ikatan, Port Moller, Copper River (silt) and Copper River Bank.

The culture prepared with the samples collected at the Copper River Bank was toxic and killed 2 guinea-pigs with symptoms of botulism on the 5th and 8th day. The type could not be determined as the animals inoculated with serum invariably showed slight indefinite symptoms and recovered.

Canada.—In December, 1920, Dr. J. C. Geiger collected, in the vicinity of Vancouver, 6 samples of soil and 9 specimens of carrots, spinach, cabbage (2), greens and beets. Two samples of soils produced weak toxins.

In September, 1921, Dr. J. C. Geiger collected 20 samples of virgin soil (?) and 4 specimens of green moss along the tract of the Seymour Water supply near Vancouver, British Columbia. The cultures gave the following results:

Green moss samples, 3 B. botulinus, type A.

Virgin soil (?), 3 B. botulinus, type A, 2 weak toxins.

In October, 1921, Dr. J. C. Geiger collected 29 samples of virgin soil from the vicinity of Victoria and Lefroy Glacier, above Lake Louise, and at the foot of Mount White, Glacier, British Columbia. The cultures repeatedly gave the following results:

Red virgin soil (20), 4 B. botulinus, type A, 1 type B, 3 weak toxins.

Gray moraine and glacier soil (4), 2 B. botulinus, type B, 1 type A and B.

Black soil from Big Beehive (7,500 feet) (5), 1 B. botulinus, type A.

Dr. Henri Larouche, Chief Division of Laboratories, Montreal, submitted, in November, 1921, 16 samples of garden and road soil collected from the following counties in the Province of Quebec: St. Eustache (2); Chemin Ste. Rose-Pont Viau; Ste. Ursule, Co. Maskinonge; Pointe St. Charles, Montreal; St. Agnes de Dundee, Co. Huntingdon; Huntingdon; Bordeau; St. Cyr, Co. Richmond; Melbourne, Co. Richmond; St. Paul l'Ermite; L'Epiphanie; St. Leonard de Port Maurice; St. Michel; St. Jacques l'Achigan; Rawdon. The cultures were all nontoxic.

Dr. Frank T. Shutt, Dominion chemist, Central Experimental Station, Ottawa, kindly submitted, in December, 1921, 16 carefully collected and packed samples of virgin and manured soils procured from 8 provinces of Canada. The cultures furnished the following results:

Virgin soil, Charlottetown, Prince Edward Island, 1 B. botulinus, type A.

Manured soil, Napan, Nova Scotia, 1 B. botulinus, type A.

Virgin soil, Cape Rouge, Quebec, 1 B. botulinus, type A.

Virgin soil, Ottawa (C. Exp. Stat.), Ontario, 1 B. botulinus, type A.

Manured soil, Ottawa (C. Exp. Stat.), Ontario, 1 B. botulinus, type B.

The samples of manured soil, Charlottetown, Prince Edward Island; virgin and manured soil, Fredericton, New Brunswick; virgin soil, Nappan, Nova Scotia; manured soil, Cape Rouge, Quebec; virgin and manured soil, Brandon, Manitoba; Invermere, British Columbia, and Lethbridge, Alberta, were apparently free from spores of B. botulinus.

Summary: 61 virgin soil, 14 type A, 3 type B, 1 type A and B, 5 weak toxins; 30 cultivated or manured soil, 1 type A, 1 type B, 2 weak toxins; 9 vegetables, negative.

Total: 100 samples with 20 (27), or 20 (27.0) %, positive cultures.

The laboratory findings are summarized in table 1.

TABLE 1

SUMMARY OF LABORATORY FINDINGS

	Locality	Specimens	Total Number of Specimens Examined	Number of Toxic Cultures	Untyped Cultures	Type A	Type B	Type A and B	Percentage of Positive Samples
	ALASKA								
192	Various stations.......	Soils	7	1	1	—	—	—	—
	CANADA								
92	Vancouver, B. C.	Soils (6); vegetables (9)	15	2	2	—	—	—	—
187	Vancouver, B. C.	Soils (20); green moss (4)	24	8	2	6	—	—	25.0
200	Glacier, Lake Louise, B. C.	Soils	29	12	3	5	3	1	31.0
213	Province of Quebec....	Soils	16	—	—	—	—	—	—
215	Charlottetown, P. E. I.	Virgin and manured soil	2	1	—	1	—	—	—
	Fredericton, N. B.	Virgin and manured soil	2	—	—	—	—	—	—
	Nappan, N. S.	Virgin and manured soil	2	1	—	1	—	—	—
	Cape Rouge, Quebec...	Virgin and manured soil	2	1	—	1	—	—	—
	Ottawa, Ont.	Virgin and manured soil	2	2	—	1	1	—	—
	Brandon. Man.	Virgin and manured soil	2	0	—	—	—	—	—
	Invermere, B. C.	Virgin and manured soil	2	0	—	—	—	—	—
	Lethbridge, Alberta....	Virgin and manured soil	2	0	—	—	—	—	—
	Total: Alaska........	....	7	1	1	0	0	0	
	Canada.......	....	100	27	7	15	4	1	
			107	28	8	15	4	1	

It has been pointed out that the number of specimens secured from Alaska is insufficient to warrant any definite conclusions. The coast soil of the Aleutian Archipelago is apparently free from spores of B. botulinus. One specimen collected from the bank of the Copper River produced on 2 occasions weakly toxic cultures which could not be typed. This river drains the Nuzotin and Wrangel Mountains, or a part of the Cordilleran system. B. botulinus may theoretically be expected in these soil stratums, and it is most unfortunate that only 2 specimens have been collected from this region. Future studies should consider the areas along the Copper and Yukon Rivers. Tests should be made, particularly in the mountains around Mt. Allen and Mt. Kimball. Until these examinations have been made, the question whether or not B. botulinus may play a rôle in the canning of Alaskan salmon cannot be answered.

Twenty-seven, or 27%, of the 100 specimens collected in Canada furnished toxic cultures. In 20 cultures, B. botulinus, type A, as well as Type B, were repeatedly demonstrated. This anaerobe is widely distributed in the Dominion, although the Western section, namely, British Columbia and particularly the Canadian Rockies, furnishes the highest percentage of positive cultures. Attention is called to the isolation of B. botulinus, type A and type B, from glacier and mountain soil, around Lake Louise. These findings fully confirm those made in the Glacier National Park and support the conception that B. botulinus is a common soil anaerobe of the Cordilleran Mountain system. B. botulinus, type A, predominated, although type B occurred occasionally either alone or in conjunction with type A. The soil samples containing type B consisted in two instances of typical moraine sand with little decaying vegetation. Without additional geologic information, it was impossible to decide whether the spores had been washed into the soil stratum or whether the organism had actually developed in this earth material. Judging by previous findings on Mount Rainier and in the Glacier National Park, it is not unlikely that the first stated view is the correct one.

Three virgin soil specimens from Prince Edward Island, Quebec, contained B. botulinus, type A, while of 2 manured soils from Nova Scotia and Ontario, 1 gave type A and 1 type B culture.

The negative results obtained with soils collected from the vicinity of Montreal and the eastern section of Quebec may be due to the selection of samples. It is, however, possible that the lower portion of the St. Lawrence River basin, draining the Great Lakes, harbors relatively few spores of B. botulinus and in this respect is comparable to and continuous with the American Great Lakes region, which is known to be slightly infected.

Sixty-one virgin soil samples furnished 23, or 37.7%, while 30 specimens from manured or cultivated fields gave only 4, or 13.3%, toxic cultures. Nine vegetable specimens were found free from B.

botulinus; 14, or 60.8%, of the toxic virgin soil specimens contained B. botulinus, type A. These findings are in accord with the observations made in the United States, and it must, therefore, be concluded that B. botulinus occurs also in Canada in an ubiquitous distribution.

CONCLUSIONS

B. botulinus has not been demonstrable in the coast land soil of the Aleutian Archipelago.

B. botulinus, type A, and occasionally type B, has been cultivated in moraine, glacier and mountain soil collected around Lake Louise in the Canadian Rockies.

Soil samples obtained from the provinces of Prince Edward Island, Nova Scotia, Quebec, Ontario and British Columbia have also furnished positive cultures.

THE OCCURRENCE OF THE SPORES OF B. BOTULINUS IN BELGIUM, DENMARK, ENGLAND, THE NETHERLANDS AND SWITZERLAND. VI *

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From the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco

Botulism has been recognized in Germany since 1735, but Bitter [1] wrote in 1921 that all attempts made in Europe to locate B. botulinus in nature had completely failed. In 1919 Semerau and Noack [2] expressed the opinion that the anaerobe occurred probably in regions or districts which from their geological or climatological location or incidental to contact with animal life and plant growth offered an environment suitable for multiplication and persistence. With the exception of the studies of van Ermengem [3] and Bitter, no data were available to support the hypothesis of Semerau and Noack. In view of the American findings, it suggested itself to examine a number of soil specimens collected in European countries in which human botulism had occurred. Such an undertaking was also prompted by a desire to compare the B. botulinus strains of the Old World with those of the New World. It can be stated without any fear of contradiction that nothing definite is known with regard to the various strains of B. botulinus which have been isolated in Europe. The early descriptions of van Ermengem and Leuchs [4] have with some slight modifications been copied by most of the recent writers. Moreover, the original strain of van Ermengem has been lost and fourteen so-called cultures of B. botulinus received from Germany during the last year have been found nontoxic, containing B. sporogenes or B. centro sporogenes. The only evidence which indicates that B. botulinus, type B or type A, is encountered in Germany is furnished by these observations: 1. The antitoxin prepared by the Institute of Infectious Diseases, Berlin, and supplied to us by Prof. Claus Schilling neutralizes the toxin of every American type B strain. 2. The Lister Institute of

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Received for publication, July 26, 1922.

* This study was aided by grants from the National Canners Association, the Canners League of California and the California Olive Association.

[1] Ergebn. d. allg. Path. u. path. Anat., 1921, 19, II, p. 762.

[2] Ztschr. f. klin. Med., 1919, 88, p. 304.

[3] Ztschr. f. Hyg. u. Infektionskrankh., 1897, 26, p. 1.

[4] Ibid., 1910, 65, p. 55.

Preventive Medicine forwarded for comparison 2 cultures of B. botulinus originally received by them from the Institute of Infectious Diseases, Berlin. One culture (No. 95) was nontoxic, while the other (No. 94) furnished a pure growth of B. botulinus, type A, which is identical with the 70 American type A cultures. It has been impossible to determine whether this culture is a descendant of the original one isolated by Gaffky [5] from the white beans responsible for the Darmstadt outbreak. In 1910 it had been demonstrated by Leuchs [4] that the strain isolated by Gaffky differed immunologically from van Ermengem's original culture. On a comparative basis it appears not unlikely that Gaffky's culture was identical with what is known today as B. botulinus, type A, and if so is probably the only type A culture which has been isolated in Europe. It was expected that the examination of soil specimens from Europe might possibly confirm or enhance this interpretation.

Besides Germany, a number of European countries have reported outbreaks of botulism. For example, van Ermengem [6] described 2 outbreaks which occurred in Belgium, 1 at Ellezelles in Hainau, and 1 at Jseghem in West Flanders. The observations reported by Madsen [7] and Hoeg [8] dealt with intoxications which followed the consumption of spoiled fish in Denmark. One outbreak each has been reported from the Netherlands and Switzerland. Unfortunately Blattmann,[9] who described cases of botulism due to smoked ham in Switzerland, failed to mention the exact location of the outbreak. One botulism observation was made in England [10] in 1860, and one in France [11] in 1875, in which it was stated that the poisonous sausages had been prepared in England. However, according to Savage [12] "botulism is unknown, or at least unrecognized," in England. This statement, made by the best known English authority on food poisoning and food infections, prompted the desire to examine soil specimens from England. On account of the supposed absence of botulism it was suspected that the soil of this country contained either none or, at most, very few spores of B. botulinus. Furthermore, by analogy with conditions in the United States it was reasoned that the heavily populated areas of the British Isles and of Europe would harbor few spores on account of the century-old manuring and tilling of the soil, and if present in such an environment that only type B would probably be encountered. That this assumption is correct is apparently proved by the findings reported in the next paragraph.

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[5] Lafar's Handbuch d. technisch. Mykologie, 1905-08, 2, p. 453.

[6] Handb. d. pathog. Mikroorganis., 1912, 4, p. 918.

[7] Centralbl. f. Bakteriol., I, Ref., 1905, 37, p. 373.

[8] Hospitalstid., 1915, 58, p. 300.

[9] Cor.-Bl. f. Schweiz. Aerzte, 1909, 39, p. 18.

[10] Brit. and For. Med., Chir. Rev., 1860, 25, p. 142.

[11] Ann. hyg. pub., 1875, 43, p. 472.

[12] Food Poisoning and Food Infections, Cambridge, 1920, p. 8.

Experimental Data

The technic employed for the study of the European soil specimens was identical with that reported in papers I, II, III, and IV of this series. This bacterio-geographical study would have been impossible without the hearty cooperation of several colleagues and friends. It is a great pleasure to acknowledge and to express again the sincerest thanks of the Hooper Foundation for the kind assistance rendered.

The observations made on 165 specimens are herewith briefly reported.

Belgium.—Through the assistance of Dr. Paul Fabry, exchange student at the University of California, 3 samples of soils were procured from Prof. E. Malvoz, at Liège, Belgium. Two cultures contained B. botulinus, type B.

Denmark.—Dr. H. O. Schmit-Jensen of Prof. C. O. Jensen's laboratory in Copenhagen, collected and forwarded in collaboration with the Lime Control Department of the Statens Planteavls Laboratorium, in June, 1921, 54 samples of soils, compost, etc., from Copenhagen and different parts of Denmark. The origin and character of the specimens are briefly as follows:

Eight garden soils [serum laboratory, botanical garden (2), Söborg, Gjentofte, Endrup, Tröröd, Copenhagen].

Twelve field soils [serum laboratory, experiment field, celery field, night soil, cucumber field, cabbage field (2), Söborg, Gjentofte, Emdrup, Tröröd bean field and leek field; all near Ullerup, Amager].

Four sewage discharging berths.

Five composts, 1 roots and leaves, etc.

Twenty-five soils from Nordby; Sorvad; Risby, Snesere; Haslund, pr. Randers; Sønderby; Borre, Moen; Saxild, Odder; Ll. Karleby, Roskilde; Hornskov, Sjørslev; Riisgaard, Grønbaek; Hauerbaek, Kjellerup; Ullerup, pr.Hurup; Baech, pr. Vonsbaech; Sommersted; Fodby, Fodby; Simonsgaard, Rutsker; Spagergaard, Nylars; Nøore, pr. Rødby; Alsonderup, pr. Hillerød; Heldumboel, Lemvig; Aistrup, pr. Sulsted; Gravlev, Støvring; Birkely, pr. Ulsted; Arvelund, Nakskov; Hammerhus, Tørring; Aale.

The following cultures proved toxic on repeated examination:

Compost (3 yrs. old) Horticulture "Nykastrupgaard, Amager, B. botulinus, type B.

Soil from Aistrup, pr. Sulsted, B. botulinus, type B.

Soil from Birkely, pr. Ulsted, B. botulinus, type B.

A sample of remnants of roots and leaves in Kongelunden, Amager, near Copenhagen, soil from Hammerhus, Tørring, from Sorvad and from Hornskov, Sjørslev, produced weak toxins.

Summary: 8 garden soils, none; 37 field soils, 2 type B, 3 weak toxins; 4 sewage discharging fields, none; 5 compost, roots and leaves, etc., 1 type B, 1 weak toxin.

Total: 54 samples with 3 (6) or 5.5 (11.1) %, positive cultures.

England.—Dr. William G. Savage, County Medical Officer of Health, Somerset County Council, Weston-super-Mare, kindly organized the collection and shipment of 64 samples of soil from the following localities in England: Colchester, Rhondda, Durham City, Wolingham, Shincliffe, Shotley Bridge;

Penycae Ruabon, Eastbourne; Ealing; Gloucester; Brighton; Shrewsbury; Timperley (Cheshire); Derbyshire; Isle of Wright; Breinton (Hereford); Weston-super-Mare.

The following samples furnished toxic cultures:

Surface soil near wood well enclosed by trees, at foot of the South Downs, near Eastbourne—B. botulinus, type B.

Soil from a heavy manured garden which had had road sweepings and horse manure dressings each year; 1 ft. deep in Ealing—B. botulinus, type B (surface soil sample of the same place, negative).

Surface soil on hillside, manured 1920, growing oats, South Downs, Brighton —B. botulinus, type B.

Soil 1 ft. deep in garden (kitchen), manured regularly with cow dung and horse manure, Shottle Hall, Derbyshire—B. botulinus, type B.

Surface soil from garden used for growing vegetables, heavily manured with farm manure each year and produces prolific crops, Breinton, near Hereford—B. botulinus, type B.

Surface soil from a field growing wheat at Shincliffe, and surface soil from sewage farm at Shotley Bridge, near Durham, produced weakly toxic cultures.

Summary: 47 garden and orchard soils (mostly manured), 4 type B, 2 weak toxins; 7 field soil, 1 type B, 1 weak toxin; 7 virgin soils, negative; 3 sewage farms, 1 weak toxin.

Total: 64 samples with 5 (9) or 7.8 (13.1) %, positive cultures.

The Netherlands.—Prof. D. A. de Jong, University of Leiden, Holland, furnished in March, 1922, 10 samples of soil which had been collected in Zeeland, Zuidland, Gelderland, Groningen, Utrecht, Drenthe, Friesland, Noord Holland, Noord Brabant and Limburg.

One specimen from Gelderland and one from Drenthe furnished B. botulinus, type B. The cultures prepared with the samples from Zeeland, Zuidland, Friesland and Noord Brabant were toxic. The filtrates produced typical botulism symptoms on feeding to guinea-pigs and mice, but neither type A nor type B nor a polyvalent antitoxin protected the animals against the intoxication.

Summary: 10 samples of soil, field or garden, 2 type B, or 20% positive cultures.

Switzerland.—Through the assistance of Prof. Dr. R. Burri, Schweizerische Milchwirtschaftliche und Bakteriologische Anstalt, Bern-Liebefeld, the laboratory received:

Sixteen samples of soil and dirt (meadows treated with liquid manure, 4; treated with cow's manure, 4; garden soil treated with cow's manure, 4; street dust, 2; and virgin soil from Alpine meadows of Canton Tessin and Bern, 2).

Eighteen specimens of vegetables (white cabbage; savoy, 2; white turnip, 2; dwarf peas; cauliflower, 2; white orach, preserved peas, yellow turnip, red beets, potato roots, silver beets, salad beets, Brussels sprouts; salad treated with liquid manure, 2).

The following specimens furnished toxic cultures:

Surface soil from meadow treated with liquid cow's manure, 1 B. botulinus, type B; 2 surface soil from cultivated field treated with cow's manure, 1 B. botulinus, type B, 1 weak toxin; 3 garden soil treated with cow's manure, 1 B. botulinus, type B; 1 salad from Köniz, treated with liquid manure, 1 B. botulinus, type B; 1 cauliflower, 1 B. botulinus, type B; 1 silver beets (Silber Mangold), 1 B. botulinus, type B; 1 red beets and 1 potato roots, 2 weak toxins; 1 Brussels sprouts, B. tetani.

Summary: 4 meadow soils treated with cow's manure, 1 type B; 4 cultivated fields treated with cow's manure, 1 type B, 1 weak toxin; 4 garden soil treated with cow's manure, 3 type B; 2 Alpine meadow soils, negative; 2 street dust, negative; 18 vegetable specimens from manured gardens, etc., 3 type B, 2 weak toxins.

Total: 34 samples with 8 (11) or 23.5 (35.2) %, positive cultures.

The essential results detailed in the preceding paragraphs are briefly sumrized in table 26.

TABLE 1

RESULTS OF EXPERIMENTS WITH SOILS FROM VARIOUS EUROPEAN COUNTRIES

Country and Locality	Specimens	Total Number of Specimens Examined	Number of Toxic Cultures	Total Number of Typed Cultures	Type B	Untyped	Percentage of Total Toxic Cultures	Percentage of Typed Cultures
Belgium:								
Unknown	Soils	3	2	2	2	—	—	—
Denmark:								
Copenhagen-Amager	Garden soil (8); field soil (12); sewage berth (4); compost (5)	29	2	1	1	1	6.8	3.4 } 5.5
Different parts of the country	Field soils	25	4	2	2	2	16.0	8.0
England:								
Different parts	Garden and orchard soil (47); field soil (7); virgin soil (7) and sewage farms (3)	64	9	5	5	4	13.1	7.8
Netherlands:								
Different parts	Meadow soil	10	6	2	2	4	(60.0)	(20.0)
Switzerland:								
Vicinity of Bern and one Canton Tessin	Soils (16) and vegetables (18)	34	11	8	8	3	35.2	23.5
Total		165	34	20	20	14	20.6	12.7

A total of 165 soil and vegetable specimens have been examined. Thirty-four, or 20.6%, of the samples furnished toxic cultures, but only 20, or 12.7%, of them have been identified as toxins of B. botulinus, type B. It must be stated at once that the cultures prepared with the European specimens exhibited one striking peculiarity, namely, the toxins generated in peptic digest-liver broth and injected into guinea-pigs in 2 c c amounts failed to kill in less than 48 hours. In fact, most of the toxins were not fatal until 3 to 4 days had passed. Previous experience had taught that these weakly toxic cultures were the probable result of the presence of relatively few heat-resistant spores. This interpretation was further supported by the observation, repeatedly made, that the heating of these soil suspensions at the temperature of the Arnold sterilizer for more than 15 minutes invariably produced nontoxic enrichment cultures. In the experiments described in previous papers this had been shown to be the case only when the original number of spores in the soil was a small one. Isolation and purification of B. botulinus from European soil samples were therefore found exceedingly difficult. Only one strain has thus far been enriched sufficiently to insure an isolation of a pure culture.

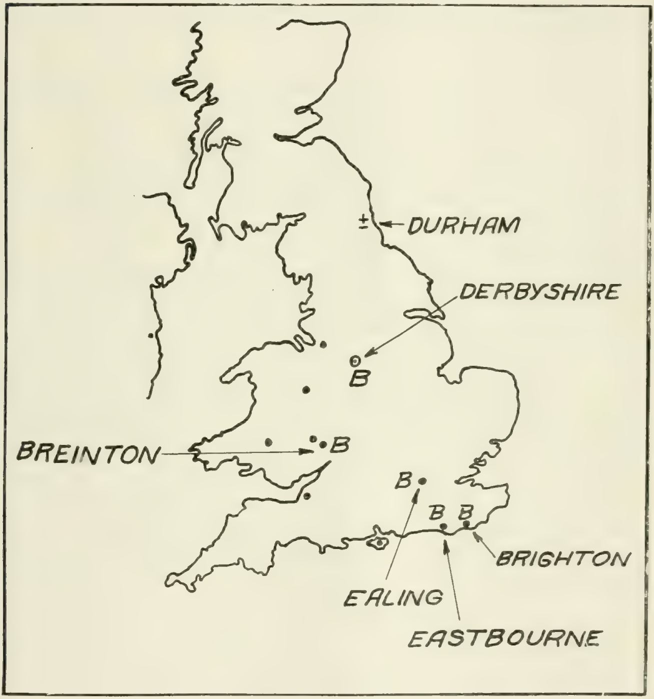

Chart 1.—Distribution of B. botulinus in England

The data secured with European field specimens confirm in a surprising manner the findings made in the United States, namely, that when type B is isolated the number of spores in the soil and its products is relatively small. Extensive cultivation suppresses B. botulinus and apparently eliminates type A. Every statement or conclusion which has been made in the discussion dealing with the telluric distribution of B. botulinus in America can, therefore, in a general way, be applied to the conditions in Europe.

Only 3 of the 54 Danish samples contained B. botulinus, type B. This survey is sufficiently extensive to permit the conclusion that B. botulinus is rarely encountered, and then only in field soil or in composts. Garden or field soil heavily polluted with sewage gave nontoxic cultures.

From a geographical standpoint, B. botulinus is widely distributed in England, as indicated by chart 1. Considering, however, the fairly large collection of specimens studied, it must be concluded that the number of organisms is relatively small, because of 64 samples only 4 garden soils and 1 field soil contained B. botulinus, type B. One specimen from Breinton produced a potent toxin, and the organism was enriched in a fair state of purity. Cultures made from virgin soil samples were free from B. botulinus and allied anaerobes, as, for example, B. sporogenes, Vibrion septique, etc. Attention is again called to the absence of B. botulinus in the soil from sewage farms.

Six, or 60%, of the 10 soil specimens obtained from The Netherlands produced toxic cultures. Two cultures contained B. botulinus, type B, toxins. The purification of one strain is expected in the very near future. Four cultures killed guinea-pigs with characteristic symptoms on feeding (2 c c) in less than 20 hours, but neither type A nor type B, nor a polyvalent antitoxin protected the animals against this toxin. The repeated demonstration of a botulinus-like toxin in anaerobic enrichment cultures prepared with heated soil specimens suggests two questions:

1. Does the soil of The Netherlands harbor an anaerobe similar to that isolated by Bengtson [13] in the United States from the larvae of Lucilia Caesar?

2. Are all clinical cases of botulism in Europe really caused by the poison of the same toxicogenic anaerobe which has been repeatedly isolated and studied in America?

These questions can be solved only when a specific antitoxin against the anaerobe of Bengtson is available. Insurmountable difficulties have been encountered in the numerous attempts to isolated this toxin-producing anaerobe from the enrichment cultures of the Dutch soil samples. It is, of course, known that the colonies of the anaerobe of Bengtson in mixed deep agar cultures resemble closely those of other nontoxic anaerobes or facultative aerobes. Several hundred colonies have been picked without meeting a toxic strain. These studies are being continued. Apropos of the question of whether or not all clinical cases of B. botulism in Europe are caused by the same anaerobe as that isolated in America, the following report of Bitter[14] is of interest. He isolated in 1918 and 1919, from a pickled herring and a spoiled ham, 2 strains of an anaerobe, which he identified on toxicological, morphologic and biologic grounds as those of B. botulinus. An attempt to classify these strains by means of antitoxin furnished by the Institute of Infectious Diseases, Berlin, was not successful. In the opinion of Bitter this failure must be attributed in all probability to the deterioration of the serums. He did not note the least protective action against the toxins of his strains. In the light of the observations made in this laboratory this statement opens up a new and important aspect. One of the antitoxins used by Bitter is identical with that used by us, and it has been furthermore established that this antitoxin neutralizes the toxin of all the American type B strains. The failures of Bitter may, therefore, be the result of two conditions: (a) the serums used by him were really deficient in antitoxin, or (b) the cultures contained neither a type A nor a type B toxin but an immunologically new poison. Whether this toxin is that of the Bengtson's anaerobe or possibly a B. botulinus, type C, cannot be determined from the description of his bacteriologic analysis. It is stated that his strains are highly proteolytic. The anaerobe isolated from Lucilia Caesar lacks this property, and it is not unlikely that Bitter's cultures were impure. The observations made previously on four untypable toxic cultures of Dutch soil prompt these hypotheses. They deserve the earliest attention of investigators interested in the etiology of the clinical picture generally designated as botulism.

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[13] Public Health Rept., 1922, 37, p. 164.

An analysis of the cultural results made on Swiss soil and vegetable specimens reveals a high percentage of positive cultures. Eleven, or 35.2% of the 34 samples produced toxic cultures, and 8, or 23.5%, contained B. botulinus, type B. Soil or vegetables derived from manured meadows, cultivated fields or gardens furnished the spore-containing samples. Two virgin soil specimens gave nontoxic cultures. This number is obviously inadequate to venture a comparison with the conditions observed in the United States. It is not unlikely that the soil of the Alps may harbor B. botulinus quite frequently. The factors which govern the distribution of B. botulinus in California are probably duplicated in Switzerland and may explain the high percentage of positive samples collected. Experiments are contemplated to verify these statements in the very near future.

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[14] Ref. 1, p. 791.

The cultural analysis of soil and vegetable specimens obtained from Belgium, England, The Netherlands and Switzerland has definitely shown that B. botulinus, type B, exists in the Old World. This anaerobe has been found in countries in which human botulism is rare or unrecognized. As a rule, the spores are decidedly less numerous in a European than in an American soil specimen. Unfortunately, the process of purification of 2 strains has not advanced sufficiently to permit the testing of the heat resistance of the spores. However, a number of observations made incidental to the isolation of this anaerobe from toxic enrichment cultures have indicated that the spores fail to survive temperatures of 100 C. for more than from 10 to 15 minutes. Exposure of from 25 to 50 gm. of European soil containing the spores of B. botulinus to the temperature of boiling water of from 20 to 30 minutes produced nontoxic enrichment cultures. On the other hand, the resistance of these soil spores to temperatures below 100 C. is probably greater than is generally assumed from the studies of van Ermengem,[6] who found that the spores of his strains withstood a temperature of 85 degrees for less than 30 minutes. Several soil specimens from Belgium, England, The Netherlands and Switzerland have been heated for 2 hours at 85 C., and repeated tests have furnished toxic enrichment cultures.

The heat resistance of the spores of B. botulinus has apparently little or no connection with the occurrence of human botulism in Europe. According to Bitter, only 2 outbreaks are known to have followed the consumption of canned foods—one due to home-canned string beans and one due to commercially packed burbot. About 68 of the known outbreaks in Belgium, Denmark, Germany and Switzerland have been caused by food which had not been subjected to severe processing temperatures. It is also pointed out that the prevalence of botulism in country districts is frequently due to inadequate preservation incidental to the careless and insanitary treatment to which the raw material is subjected by the rural population. Home slaughtering and preservation of pork products in the form of sausages is so widely practiced in Europe that it is not at all surprising to find that about one-half of the botulism outbreaks are caused by this type of food. Accidental contamination of these products with infected soil occurs only under the most insanitary conditions of rural districts. It must therefore be expected that human botulism due to meat, fish or animal products occurs mainly in the regions in which the B. botulinus soil index is relatively high.[15]

CONCLUSIONS

B. botulinus, type B, has been demonstrated in soil and vegetable specimens collected in Belgium, Denmark, England, The Netherlands and Switzerland.

B. botulinus, type A, has been found consistently absent.

The spores are widely distributed, but they are neither numerous nor very resistant to heat.

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[15] Since this paper has been written, one of the authors (K. F. M.) has investigated an outbreak of botulism due to commercially packed potted meat at Loch Maree, Scotland. The wild duck paste was understerilized, exhibited no signs of spoilage and contained, according to the careful studies of Mr. Bruce White of Bristol University, B. botulinus, type A., and its toxin.

Dr. J. Williamson Tocher, Aberdeen, Scotland. has isolated B. botulinus, type A., from the spleen of a horse, which suffered from "grass sickness." Dr. J. B. Buxton of the Wellcome Research Laboratory has also demonstrated the toxin of B. botulinus, type A., in the blood serum of horses which have recovered from the same disease.

During the last 3 years, 2 outbreaks of botulism due to smoked ham have occurred in Switzerland. The toxin has been demonstrated but not typed in one instance.

THE OCCURRENCE OF THE SPORES OF B. BOTULINUS IN THE HAWAIIAN ISLANDS AND CHINA. VII *

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The predominance of B. botulinus, type A, in the Pacific Coast States is in striking contrast to that of type B in the Atlantic States, England and Europe. These differences in the distribution of types cannot be attributed to any topographic factors but coincide amazingly with the density of the population and agricultural activity. It naturally suggests itself to compare these observations with those found in specimens collected in other portions of the Western Hemisphere and in certain parts of the Eastern Hemisphere. The Hawaiian Islands have been chosen on account of their mountainous surface and their volcanic origin. The soil is very fertile, being formed by the disintegration of volcanic rocks and the decay of vegetable matter. These islands possess geologically a soil stratum which in all respects is very similar to that of the Pacific Coast and Sierra Nevada mountains. Furthermore, there is considerable agricultural activity in form of cane sugar and pineapple plantations.

The distribution of B. botulinus on the Asiatic continent has been studied on specimens obtained from China. This republic is heavily populated and is one of the most important agricultural countries of the Far East. Fertilization with sewage and animal manure has been practiced for centuries. The soil is known to contain B. tetani and other pathogenic anaerobes. A study of the soil specimens offers, therefore, data of comparative value.

Experimental Data

Three series of specimens have been examined by the technic employed in similar studies. Guinea-pigs as well as mice have been used for the identification of toxic enrichment cultures.

Hawaiian Islands.—Through the courtesy of Mr. R. I. Bentley, President of the California Packing Corporation, 23 samples of soil and vegetables were procured from Wahiawa, of the Island of Oahu, in April, 1921. Cultures were made of the following specimens:

Received for publication, July 26, 1922.

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* This study was aided by grants from the National Canners Association, the Canners League of California and the California Olive Association.

Five soil samples.

Eighteen specimens of banana roots, pineapple roots with manure (4); grass, papaia roots, tomato plants, parsley, radish, turnip, beet, strawberries, papaia fruit, banana, mulberries, cabbage, tops and roots of strawberries.

One sample of pineapple root, papaia root and mulberries produced weakly toxic cultures.

In January, 1922, one of us (P. S.) collected and made cultures of 16 additional samples of soil and feeds from the Island of Oahu. The cultures gave the following results:

One soil—Algarobagrove—not cultivated, about 150 ft. from a stable. B. botulinus, type B.

One surface soil, 6 inches from surface (equine botulism outbreak?), Farm I., B. botulinus, type B.

One deep soil, 1 ft. down near stable, farm 2, B. botulinus, type B.

One surface soil, same place, B. botulinus, type B.

One virgin surface soil—Tantalus, about 6 inches deep, B. botulinus, type A.

One virgin soil side road, 2 ft. down "Round Top," B. botulinus, types A and B.

One soil, same place suface soil, B. botulinus, type B.

One good grade rice straw, weak toxin. Manure pile, drainage, 200 ft. from stable, rice straw on farm 1, garden soil on "Round Top," lava deposits, soil from Tantalus, wet and dry tarrow patch in Manoa Valley (8), all negative.

Summary: 17 soil cultivated or manured, 5 type B; 2 virgin soil in mountain region, 1 type A and 1 type A and B; 20 vegetables and feed, 4 weak toxins.

Total: 39 samples with 7 (11) or 17.9 (28.2) %, positive cultures.

China.—In May, 1922, the laboratory received from Dr. Carl Ten Broeck, Peking Union Medical College, China, 52 specimens of soil collected from the following provinces and towns:

Thirty-two Chilili: Peking, 18 (near grave, 3; cultivated fields, 9; ground covered with trees, 2; temple ground near Lo TzLung AnTom, 1; cleared ground, 3). Near Temple Heaven, 14 (cleared ground, 6; ground covered with trees, 8).

Twenty Shansi: Near town of Kao Chia Chuang, 12 (cultivated field, 1; ground covered with trees, 8; freshly cleared field never cultivated, 1; cleared ground, 1; open forest, 1). Near town of Tzi Lao, 4 (hillside forest, 1; clear ground covered with trees, 3). Near town of Chang Kou, 1 (cleared ground,1). Near town of Ping Ting Chow, 2 (cultivated field, 2). Near town of Le Ping, 1 (cultivated garden, 1).

The cultures gave the following results:

Three cultivated field (no decayed vegetable matter present), Peking, 3 type B.

One temple ground near the door of Lo Tz Lung An Tom, Peking, 1 type B.

One near grave, 1 weak toxin.

Three ground covered with trees, near town of Temple Heaven, Peking, 2 type B, 1 weak toxin.

Two cleared ground, near town of Temple Heaven, Peking, 1 type B, 1 weak toxin.

Three ground covered with trees, near town of Kao Chia Chuang, Shansi, 1 type B, 2 weak toxins.

Two cleared ground, no decayed vegetable matter present, near town of Chang Kou, Shansi, 1 type A and 1 type B.

One hillside forest (open), near town of Tzi Lao, 1 type B.

One freshly cleared field, some roots, etc., Kao Chia Chuang, Shansi, 1 type B.

Two ground covered with trees, near town of Tzi Lao, 2 type B.

One cultivated field garden near town of Le Ping, 1 weak toxin.

Summary: 12 cultivated fields with and without decayed vegetable matter, 3 type B, 1 B. tetani; 21 grounds covered with trees, some without decayed vegetation, 5 type B, 3 weak toxins, 1 type A; 11 cleared ground with and without decayed vegetation, 2 type B, 1 weak toxin; 3 near grave, 1 weak toxin; 1 hillside forest, abundant vegetation, 1 type B; 1 open forest on steep hill (large amount of decayed pine needles) ———; 1 cultivated garden, probably manure, 1 weak toxin; 1 temple ground, 1 type B (weak); 1 freshly cleared field, decayed vegetable matter present, 1 type B.

Total: 52 samples with 14 (20) or 26.9 (38.4) %, positive cultures.

Eleven, or 28.2%, of the 39 soil and vegetable samples collected in the Hawaiian Islands produced toxic enrichment cultures. The toxin of B. botulinus was identified in 7, or 17.9%, of the samples by antitoxin neutralization. Five cultures contained B. botulinus, type B. while one was neutralized by a type A and another by a polyvalent antitoxin. Four cultures prepared from fruits, roots and rice straw yielded weak toxins. The spores B. botulinus, type B, were quite frequently encountered in the soil of the Island of Oahu. Surface as well as deep soil, and virgin as well as cultivated soil, carried the organism. B. botulinus, type A, was found alone only in one virgin soil specimen, but in another instance was present with type B in a surface mountain soil. Type B is the principal toxicogenic anaerobe. This observation apparently confirms our opinion that type B exists primarily in fertile soils which are favored by the decay of vegetable matter and abundant rainfall. The percolating rain water is probably responsible for the presence of B. botulinus spores in the deeper layers of the soil. Conditions favorable for generation of toxins in moldy straw, etc., are unquestionably present, and it is therefore not surprising to learn that one outbreak of equine botulism has been observed on the premises of the U. S. Aviation Field on Oahu. The laboratory studies presented in this paper have demonstrated the presence of the anaerobe in the rice straw and the soil of the farm on which the outbreak occurred. Major James S. Simmons, M. C., isolated the B. botulinus from the spleen of one of the horses. It is most unfortunate that at the time of the outbreak the suspected hay was not studied for the determination of the presence of B. botulinus toxin. Human cases of botulism have thus far not been recognized in the Hawaiian Islands.

The observations made on the 52 soil samples procured from two coast provinces in China duplicate those of the Hawaiian Islands, except that the percentage of toxic cultures is somewhat higher, namely, 38.4%. One enrichment culture prepared from a specimen of soil derived from cleared grounds near Kao Chia Chuang was highly toxic and contained B. botulinus, type A. B. botulinus, type B, obtained in all the other specimens, is geographically widely distributed, but judging from the toxicity of the cultures, it is quite evident that the soil contains relatively few spores. The majority of toxins were fatal to guinea-pigs in not less than 36 to 72 hours. Grounds covered with trees, some showing decayed vegetation, yielded the largest percentage of toxic cultures. A few soils obtained from forests, etc., harbored the same type, and it is impossible to offer any final explanations for the predominance of type B in the samples examined. The evidence strongly indicates that this type is indigenous to the lowland of China. A study of soils collected in the Khingan Mountains or the interior of China, which is less heavily populated than the provinces of Chilili and Shansi, should be made in order to amplify the results thus far obtained. Geographic and telluric studies on the distribution of the various types of B. botulinus tend to emphasize one fact—the soils of the New World, and particularly the Cordilleran mountain system of North America, harbor type A, while countries with old civilizations, as, for example, China and Europe and some of the Atlantic and Middle Western States of America, contain B. botulinus, type B., either exclusively, or at least predominantly. The surveys thus far completed unquestionably offer scattered and fragmentary evidence of a distinct process of bacterial mutation, and it is along these lines that the distribution of B. botulinus should be further investigated. The problem of microbial evolution or the adaptive development of bacterial species to their environment receives in the light of the geographic studies on B. botulinus a new impetus, and it is hoped that additional work on the presence of this anaerobe in South America, India, Africa and Australia may be undertaken in the not too distant future.

CONCLUSIONS

B. botulinus, type B, is frequently found in the soils obtained from the Island of Oahu, in the Territory of Hawaii, and from the provinces of Chilili and Shansi, in China. Type A has been found only in two Hawaiian and in one Chinese soil specimen.

THE OCCURRENCE OF B. TETANI IN SOIL AND ON VEGETABLES. VIII *

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A number of enrichment cultures prepared in the course of the studies on the distribution of B. botulinus in the soil of the United States, Switzerland and China, produced on subcutaneous inoculation in guinea-pigs typical symptoms of tetanus. In every instance neutralization of the toxin was accomplished by the simultaneous injection of 1,000 units of commercial antitoxin. Unfiltered but centrifugalized cultures which caused tetanus were tested for B. botulinus toxin by feeding portions to guinea-pigs and mice. One sample of the soil from Maryland was found to contain the toxin of B. botulinus, type B, and that of B. tetani. The data, dealing with the distribution of the tetanus bacillus in 2,379 soil and vegetable samples collected in the United States, Europe and China, are the result of casual observations. No attempt has been made to study the factors necessary to demonstrate this bacillus in mass enrichment cultures. It is known that the toxicogenic capacity of B. tetani is greatly reduced in the presence of other aerobes and anaerobes, and it is quite possible that the conditions chosen for the study of B. botulinus were not always suitable for the production of a potent tetanus toxin. In order to stimulate a study of the distribution of B. tetani in nature, however, the few observations are briefly summarized in table 1.

TABLE 1

Distribution of B. tetani in Various States

Arkansas	2 uncultivated virgin soil samples
California	Horse trail on Mt. Lowe, near Los Angeles
Indiana	1 pasture and 1 vegetable (swiss chard)
Maryland	1 uncultivated virgin soil sample, together with B. botulinus, type B
Michigan	2 garden soil specimens
Minnesota	2 garden soil specimens
New York	3 virgin soils collected around Mt. Baker, near Saranac Lake
Rhode Island	1 cultivated field (corn) and on 1 spinach specimen
West Virginia	1 virgin soil specimen from the Alleghany Mountains
Wisconsin	3 soil specimens from a heavily manured celery farm near Milwaukee
Switzerland	1 vegetable (brussels sprouts) from the vicinity of Bern
China	1 cultivated field near Pekin

Received for publication, July 26, 1922.

* This study was aided by grants from the National Canners Association, the Canners League of California and the California Olive Association.

It will be noted from the data presented in this table that nine of the states of America which furnished enrichment cultures with B. tetani are topographically east of the Mississippi. No western soil material, with the exception of one (trail dust on Mt. Lowe, Calif.), produced cultures containing B. tetani. These differences are, on account of the large number of cultures involved, sufficiently striking to justify further discussion. Three hundred and ninety-seven specimens from 9 (Arkansas, Indiana, Maryland, Michigan, Minnesota, New Jersey, Rhode Island, West Virginia and Wisconsin) states mentioned in table 1 furnished 18 cultures, while 991 cultures prepared with similar samples from California, Oregon, Washington, Montana, Idaho, Nevada, and Wyoming gave only 1 culture containing B. tetani. During a study of 624 specimens which originated in California, special attention was paid to the occurrence of tetanus bacilli in mountain soil, but the negative results prompted the conclusion that the spores of B. tetani are rare in comparison to those of B. botulinus. As it is known that well-manured garden soil has yielded tetanus bacilli in the hands of various observers, it is rather surprising to find the numerous specimens of garden and manured field soil from Oregon, Washington and California free from this anaerobe. It has been suggested that these results are due to the technic employed, and in particular the method of testing the toxicity of the enrichment culture has been suspected as inadequate. In this connection it should be stated at once that the tetanus toxin in the enrichment cultures was always recognized in the course of the neutralization tests with B. botulinus antitoxin. As a rule, all 3 guinea-pigs developed tetanus. The criticism that the presence of tetanus spores cannot always be demonstrated in mixed cultures is probably true for a small number of specimens, but this must apply equally to the samples collected in the East and Middle West, as the same method was employed throughout the entire series of studies. The evidence permits, therefore, only one conclusion: B. tetani is less prevalent in the soil of western states than in that of the eastern and middle western states. It is naturally possible that permanent infestation of the regions originally free from it may occur through commercial and industrial agencies, just as certain sections of California (Sacramento delta) have become permanently infected with anthrax spores from the offal and residues washed down into streams from tanneries, hair factories and improper burial places of animal cadavers.

The demonstration of tetanus spores in garden soil and on vegetables collected in eastern territories, Switzerland and China is quite in accord with the well-known facts established many years ago by Nicolaier,[1] Bossano,[2] Bisserié,[3] Sanchez-Toledo and Veillon,[4] Rabinowitch,[5] and others. The association of tetanus spores with fertilization and manure has always been supported by an old theory derived from the French, which claims that the tetanus bacillus is a regular inhabitant of the intestines of the horse and other species of domestic animals. Even human feces have, according to Pizzini,[6] in Italy, and Ten Broeck,[7] in China, been found to harbor this anaerobe. According to the data presented in table 1, spores of B. tetani are found in virgin and uncultivated forest soil where plant material is undergoing fermentation and decay. It is, therefore, quite conceivable that this anaerobe can, just as B. botulinus, multiply in symbiotic relation with other anaerobes or aerobes, wherever protein material undergoes putrefaction. The presence of the tetanus bacillus in so many places outside the intestinal canals of animals suggests much more extensive breeding places than the animal body. Irrespective of the recent findings of Ninni,[8] who failed to find the spores of B. tetani in the soil from mountainous regions and elsewhere free from the dejecta of domestic animals, in studying B. tetani careful consideration should be given the facts established for B. botulinus. The entire problem of breeding places of all pathogenic anaerobes deserves renewed investigation in the light of the data presented in the papers dealing with the distribution of B. botulinus in nature.

CONCLUSIONS

The spores of B. tetani are frequently encountered in well manured, cultivated or garden soil, and on vegetables obtained from several states east of the Mississippi, Switzerland and China. In the United States even virgin forest soil has yielded cultures of B. tetani. The soil of the western states is relatively free from this anaerobe.

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[1] Deutsch. med. Wchnschr., 1884, 10, p. 842; Beitr. z. Aetiologie des Wundstarr Krampfes, Göttingen, 1885.

[2] Rev. de méd., 1889, 9, p. 102.

[3] Thèse, Paris, 1894, No. 126.

[4] Semaine méd., 1890, 10, p. 45.

[5] Arch. f. Hygiene, 1907, 61, p. 103.

[6] Riv. d'igiene e san. pubbl., 1898, 10, p. 170.

[7] Personal communication, and Jour. Exper. Med., 1922, 36, p. 267.

[8] Ann. d'igiene, Rome, 1920, 30, p. 756.

A SAFE METHOD FOR SECURING ANAEROBIOSIS WITH HYDROGEN. IX *

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In the course of some quantitative studies on the growth of anaerobes in various plant and animal products, one of the authors (C. C. D.) has, during the past year, employed 13 large anaerobic jars. They have been operated in accordance with the principles proposed by Laidlaw [1] and constructed according to the descriptions published by McIntosh and Fildes.[2] It is the purpose of this paper to enumerate briefly the various details and operations which have eliminated the danger of explosions. The arrangement of the equipment as used in this laboratory is shown in fig. 1.

It will be seen from the illustration that the jar is not unlike that described by Brown.[3] A few alterations in construction are indicated in the photograph and are briefly summarized as follows: (1) The wires conveying current to the heating coil are soldered [4] to short pieces of "Pyrex" glass tubing, which are then cemented into a rubber stopper (A). This method of introducing the lead wires readily permits the use of multiple stranded copper wire (as ordinary, flexible electric light cord), minimizing the chance of breakage. The wires should be looped over and tied to the side of the glass tubes on the outside of the jar top to prevent any strain from occurring at the soldered joint. (2) A shield of asbestos sheet (B) is placed over the upper surface of the coil to prevent the heat from cracking the jar top. (3) Instead of mica as used by McIntosh and Fildes, asbestos washers are placed at these points (C) to deflect the heat from the inner ends of the rubber stoppers.

In several papers,[5] it is emphasized that the anaerobic apparatus embodying the principle of Brown's jar produces excellent results, but

Received for publication. Aug. 7. 1922.

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* This study was aided by a grant from the National Canner's Association, the Canner's League of California and California Olive Association.

[1] Brit. Med. Jour., 1915, 1, p. 497.

[2] Lancet, 1916, 1, p. 768.

[3] Jour. Exper. Med., 1921, 33, p. 677.

[4] Modification of method described by McKelvy (Jour. Am. Chem. Soc., July, 1920). Surface of glass to be soldered coated with metallic platinum by dipping repeatedly in alcoholic $PtCl_4$ solution and heating to a dull red heat.

[5] Smillie: Jour. Exper. Med., 1917, 26, p. 59. Berg: Jour. Am. Vet. Assn., 1922, 14, p. 189. Gochenour: Ibid., 1922, 14, p. 301.

it is also indicated that in all the adaptations or modifications some danger of explosion still persists. The risk of igniting the hydrogen in the jar from the catalyzer itself seems to be eliminated by the use of the wire gauze employed in the construction of the capsule and electrically heated catalyzer described by McIntosh and Fildes. In a recent note, Brown[6] has, however, pointed out that an explosive mixture still exists in the jar, which may be ignited by a temporary shorting or breaking of the wires, while the circuit is closed. By operating the apparatus in accordance with the method about to be described, the writers believe that the danger of a hydrogen explosion occurring in the jar may be entirely eliminated.

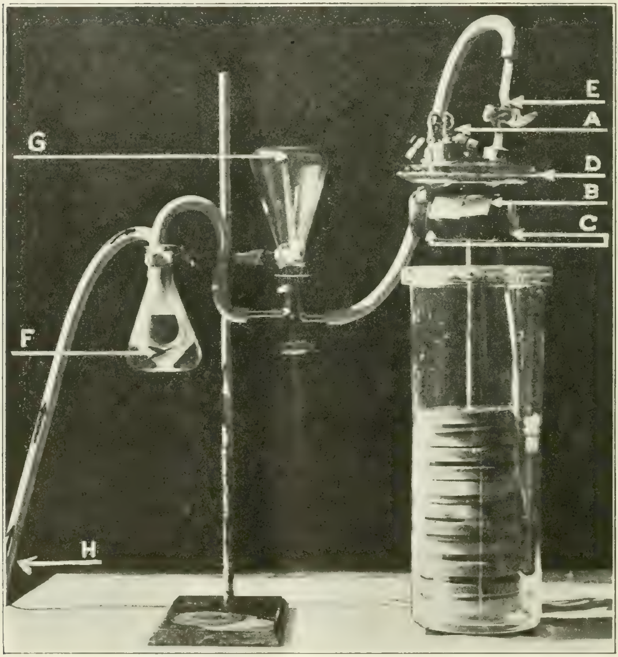

Figure 1

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[6] Jour. Exper. Med., 1922, 35, p. 467.

TECHNIC EMPLOYED IN OPERATING JAR

The flange of the jar top (D) is coated to a depth of about ¼ inch with plasticine modeling clay, which is spread to form a smooth layer of even depth. The top is pressed firmly in the clay and the jar is then evacuated through the stop-cock (E). As a rule, the plasticine joint around the top leaks considerably when the jar is first attached to the vacuum pump, but as soon as the pressure within the jar is reduced, the top will set more firmly, forcing out a ridge of plasticine. By spreading the projecting clay smoothly over the interstice between the jar and top, a tight seal may readily be obtained. Plasticine exposed to 37 C. becomes brittle, and frequent renewal is advocated. When evacuation of the jar is completed, the stop-cock (E) is closed and the jar connected to the hydrogen supply. Hydrogen is passed in under very slight pressure until the pressure within the jar reaches that of the hydrogen supply. A convenient apparatus for controlling the gas flow is shown (F, G and H) and will be described in detail in a subsequent paragraph. An electric current of suitable strength is passed through the heating coil until a satisfactory anaerobiosis is secured.[7] The jar is permitted to remain connected with the hydrogen supply during this operation, after which the stop-cock is closed and the jar disconnected for incubation.

A Kipp generator will supply hydrogen at sufficient pressure to give satisfactory results with this method. Where hydrogen can be obtained in cylinders, a much more convenient and dependable supply is afforded. A reducing valve is desirable but not absolutely necessary with the gas controlling apparatus shown in fig. 1, which consists of the following parts: (F) flask (open at top) containing a loop of pressure tube submerged in water; a slit about ¾ inch long in the rubber tube in the flask (F) functions as a safety valve; (G) inverted flask fitted with a three-holed rubber stopper, accommodating a hydrogen intake tube (short), a hydrogen discharge tube (long), and a drain tube (short) with stop-cock. H indicates the hydrogen supply. This apparatus serves as a trap to remove any water which may be carried over from the flask (F).

In practice, the "safety-valve" device also serves as a visible index of the gas flow. A rapid flow of gas is permitted until the vacuum in the jar is overcome, permitting the filling of the jar in a few seconds. When the jar has been filled, as indicated by the emission of gas at the slit, the flow of hydrogen may be reduced (until only a tiny stream of bubbles escapes) during the remainder of the operation.

If the jar is to be incubated much above room temperature, it is advisable to diminish the pressure in the jar, unless a clamp is applied to hold down the tops. The removal of a few cubic centimeters of gas with a pump suffices to prevent the top from being displaced by expansion. It is recommended that the use of a clamp to hold the top in position be avoided until it is disconnected from the electric current and the hydrogen supply. The danger incidental to overfilling or ignition is reduced to a minimum when the top is only retained by the viscosity of the plasticine gasket. This fact is strongly emphasized by an experiment in which an explosion has purposely been induced.

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[7] No experiments have been made to determine what temperature is necessary to activate the catalyzer, nor exactly how much time is needed to deoxygenize completely the jars at the temperature employed. Preliminary tests with illuminating gas have suggested the following heating capacity, which has been used with entire satisfaction: The heating coil consists of 3 feet of nichrome wire (No. 30 B. and S.). It is operated by connecting in series parallel with two 50 watt lamps on a 110 volt a. c. circuit. The current is usually left on about 20 minutes, one of the lamps being cut out 2 or 3 times after starting, to avoid unnecessary heating of the jar and contents.

It is evident that the operation of the jar described produces at the time the electric current is passed through the coil, a mixture of gases which is so deficient in oxygen as to be practically nonexplosive. This fact has been conclusively demonstrated by the following experiment:

An anaerobic jar similar to that shown in fig. 1 (except that the catalyzing unit was removed) was sealed, exhausted, and filled with hydrogen as described. An attempt was then made to ignite the gas within the jar by passing an electric current (110 volts, a. c.) through a fine steel filament within the jar. The filament fused, breaking the circuit and consequently inducing a spark of considerable intensity, but no explosion resulted. The test was then repeated, using a jar which was poorly exhausted, the internal pressure being about 30 cm. before the hydrogen was introduced. An explosion of considerable violence took place. It must be stated at once, however, that although the top of the jar was thrown upward with great force, the jar itself remained intact, and no shattering of glass occurred. Obviously, if the top had been clamped on the jar, the destructive force of the explosion would have been greatly increased.

Since the absolute safety of this method depends on the removal of a large proportion of the oxygen by evacuation, it appeared advisable to determine whether or not its application would be of value in laboratories equipped with relatively poor vacuum pumps. With this object in view, a jar was evacuated to 30 cm. pressure (which is readily obtainable with an ordinary aspirator, or water pump), and then the partial vacuum was replaced by hydrogen. This operation was repeated, after which the jar was submitted to the ignition test described. No explosion occurred.

It can be stated without any fear of contradiction that the anaerobe jar constructed according to the principles of Fildes and McIntosh[8] is the most dependable equipment, which produces regularly a satisfactory degree of anaerobiosis. Problems dealing with the quantitative estimation of bacterial growth of B. botulinus, B. tetani, and so forth, in mediums of varying composition and hydrogen-ion concentration have been made accessible by the use of these jars. Excellent growth has been obtained on the surface as well as in the depth of plain liver or milk agar plates. Papers which are in process of preparation indicate that the study of the physiology of sporulation, enzyme production and colony characteristics has been materially enhanced by the apparatus and operations described. In fact, the isolation and purification of delicate anaerobes have been greatly facilitated by the use of sheep blood agar plates in a reliable anaerobic jar.

From the foregoing, it is concluded:

1. That by means of the technic here described, dependable anaerobiosis may easily be secured in jars of the type described, with no

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[8] Brit. Jour. Exper. Med., 1921, 2, p. 153.

danger from possible ignition by the electric current employed in activating the catalyzer; and that in the absence of an efficient vacuum pump the safety of the method may be insured by "washing" the jar several times with hydrogen after a partial evacuation.

2. That by dispensing with the use of a clamp to hold the jar top in position, the danger arising from an explosion from any possible cause may be reduced to a minimum.

3. That jars constructed and operated as described are capable of producing anaerobiosis, with a negligible risk to the operator.

SOME OBSERVATIONS ON THE PATHOGENICITY OF B. BOTULINUS. X

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During the last four or five years, owing to the apparently increasing number of cases of food poisoning among human beings and animals definitely recognized as being due to the contamination of foods with B. botulinus, an intensive study has been undertaken of various phases of this question. More particularly is this true in the United States where, because of the increased use of home canned foods urged on the population during the World War by the government, there seem to have been more of these outbreaks. A study of the rapidly growing literature on this subject testifies to the fact that much important work has been accomplished in elucidating many of the problems, both scientific and practical, which confront those responsible for and interested in safeguarding the public health. It appears to the writers that one of the most important questions in this connection which has received insufficient attention concerns the pathogenicity of the organism itself when introduced into the animal body.

The study of this particular problem may properly be divided into three parts: (1) The injection or ingestion of detoxified B. botulinus spores or bacilli; (2) the injection or ingestion of the spores or bacilli together with a minute dose of B. botulinus toxin (or other substances) insufficient by themselves to produce symptoms of botulism; (3) the latency of injected or ingested spores of B. botulinus in the animal body where such spores, due to various causes, may later germinate, multiply or be freed of their toxin and produce symptoms of the disease.

The first of these conditions will concern us principally in this paper, though perhaps the third part is of equal importance, and being intimately connected with the first, it will be necessary to touch on its various phases from time to time. However, a more complete discussion of the last two problems will be reserved for a later paper.

Frequently spore-containing foods are eaten which may have been sufficiently heated to destroy the toxin but not the spores. As such foods may contain few or many viable spores, it becomes of the

Received for publication, Aug. 7, 1922.

gravest importance to increase our knowledge as to the possible pathogenicity of these detoxified spores thus consumed. In considering this possibility, we are immediately confronted by the following questions: Does the introduction of detoxified spores into the animal body produce symptoms or death from botulism? If so, how many such spores are necessary to produce these effects, and finally, what is the mechanism of the pathogenic manifestation of these organisms?

The work dealing with the effects of the injection of unheated B. botulinus cultures does not concern us for the moment, but a review of the few investigations that have been made with detoxified spores shows us that to produce toxic symptoms a larger number of spores is required when fed than when injected by other routes.

The early experiments of van Ermengem[1] would tend to demonstrate that "B. botulinus is absolutely incapable of reproducing itself in the animal body." His cultures of the various organs and contents of the intestinal tract were negative. He admits, however, the possibility of growth in the intestinal tract under proper, but unknown, conditions. Other European workers, following his investigations, came to similar conclusions. These workers did not use a medium in the production of their spores capable of giving a maximum toxicity. Later Dickson[2] stated that the toxin is never formed within the body, and he bases his conclusion on his belief that the toxin could not be formed at a temperature in excess of 28 C. Graham and Brückner[3] state that in cases of forage poisoning "a fatal intoxication was suggested by repeated failures to recover the organism from the blood in the course of fatal and experimental disease, while the brain, cord, liver, blood, spleen, mesentery, and suprarenals from 26 experimental cases of fed and subcutaneously injected animals were all negative."

Thom, Edmondson and Giltner[4] originally failed to produce symptoms of poisoning by feeding or injecting detoxified spores of B. botulinus, but later[5] these authors were successful. They caused death in 1 guinea-pig in 13 days, by the subcutaneous injection of only 30,000,000 spores of the Boise strain, though 31,000,000 spores of the Nevin strain produced no effect. In their feeding experiments, they produced death in 1 animal with 78,000,000 spores (Nevin strain) in 7 days, though subsequently from 75,000,000 to 112,500,000 spores of the same strain failed to produce any result. The Boise strain was fatal in from 5 to 7 days in doses of 180,000,000 to 192,000,000 spores. These authors also conducted numerous experiments based on the work of Bullock and Cramer[6] who injected calcium chloride at the same time as the spores, or at varying intervals thereafter, thereby reducing the time survival of their guinea-pigs. They admit that their results are irregular, but are convinced of the pathogenicity of the detoxified spores of B. botulinus. They also admit the possibility of growth in the animal body, as well as the absorption of intracellular toxin from the spores.

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[1] Ztschr. f. Hyg. u. Infektionskr., 1897, 1, p. 26.
[2] Canad. Med. Assn. Jour., 1918, 8, p. 903.
[3] Jour. Bacteriol., 1919, 4, p. 1.
[4] Jour. Am. Med. Assn., 1919, 73, p. 907.
[5] Arch. Int. Med., 1920, 26, p. 356.
[6] Proc. Roy. Soc., London, Ser. B, 1919, 90, p. 513.

During their investigation of an outbreak of olive poisoning, Armstrong, Story and Scott[7] inoculated a guinea-pig subcutaneously with a heated suspension containing "some 300,000,000 bacilli . . . and numerous spores," with no result. Heated cultures were fed with negative results, while washed spores were fatal when force fed, but not when fed on grass or meal. These authors by experiment estimated 1 olive to contain "a minimum of 1,300,000,000 bacilli, presumably spore bearers" and believe from their work with animals that "a bite of olive containing this number of viable organisms, if capable of multiplying and forming toxin in the alimentary tract, should have caused serious infection."

Burke, Elder and Pischel,[8] from a study of numerous outbreaks of botulism in man, conclude that the ingestion of toxin free spores, particularly in small numbers, does not cause this disease. Orr[9] shows that the feeding or injection of massive doses of toxin free spores produced botulism in guinea-pigs and mice. He caused the death of 1 guinea-pig in 5 days and only "weakness" in another, by feeding 90,000,000 spores. One animal died in 33½ hours after the ingestion of 200,000,000 spores. Of 2 animals injected subcutaneously with 45,000,000 and 50,000,000 spores, the first died in 249 hours and the other in 50 hours. He believes that the spores grow in the body and produce toxin which can be demonstrated by a precipitin test, as well as by direct toxicity tests of the emulsified organs. The counted spores in some of these experiments were fed on bread, which would appear to be a source of error in the estimation of the number of spores actually entering the digestive tract. The irregularity of Orr's results is similar to that obtained by Thom, Edmondson and Giltner. Geiger, Dickson and Meyer,[10] in a complete analysis of the question of botulism, more particularly in that portion of their work devoted to its epidemiology, discuss the possible pathogenicity of the detoxified spores. They imply some doubt as to the complete detoxification of the spores used by some of the other workers who produced botulism in animals. They, themselves, obtained negative results when heavy spore emulsions were mixed with the specific antitoxin, washed, and then heated at 80 C., and they suggest that further evidence should be obtained in this matter by careful scrutiny of further outbreaks before assuming that detoxified spores naturally ingested by human beings are pathogenic. Furthermore, they cite several human cases to show that those who ate the raw spoiled food died, while those who ate the same food heated showed no symptoms. However, they add that the experience gained by a study of these outbreaks neither supports nor refutes the results of these experimental investigations as the spore content of these heated foods was unknown.

Graham, Schwarze and Boughton[11] have recently shown that freshly drawn milk from artificially infected udders of cows is, under certain conditions, lethal for guinea-pigs, but the elaboration of toxin in the animal udder tissue or mammary secretion could not be established. These authors conclude from these and numerous feeding experiments that the possibility of B. botulinus toxin being excreted in the milk of lactating animals consuming contaminating feed is remote, though the contamination of milk by the feces or other sources furnishing spores of B. botulinus is not to be disregarded.

[7] Pub. Health Repts., 1919, 34, p. 2895.

[8] Arch. Int. Med., 1921, 27, p. 265.

[9] Jour. Infect. Dis., 1922, 30, p. 118.

[10] Bull. U. S. Public Health Service, in press.

[11] Am. Jour. Pub. Health, 1922, 12, p. 659.

Several of the following factors may play an important part in estimating the value of and in comparing the results obtained by different workers along these lines. It is important to know the method of determining the number of viable spores, the approximate number of vegetating forms present, the mediums from which they were obtained, their age, the methods of freeing them from their toxin; also the time elapsed between the detoxification and counting, as well as the injection, the method employed in feeding, and finally the purity of the spores after these various manipulations. Few workers mention all of these conditions or give controls for toxicity of the culture from which the spores were obtained, or for complete detoxification at the moment of injection.

As the pathogenicity of bacteria for the animal body as a rule depends on their ability to invade and reproduce themselves in the tissues, it is unfortunate that so few workers have studied culturally the organs of experimental and clinical cases of botulism. Orr[12] recovered B. botulinus from the spleen and liver. He also found the liver and brain of his experimental animals toxic for mice, and later records a few positive findings of B. botulinus in the liver and spleen together with entirely negative results in some of his fatally infected animals injected subcutaneously and fed large doses. Dickson[13] recovered the organism from the spleens of a few of his animals injected intravenously and van Ermengem, among the first, remarked that B. botulinus could be recovered only from the organs of animals in which a large quantity of spore material had been injected by this route. The same worker,[14] as well as Ornstein[15] and Graham,[16] also reports having found B. botulinus in the spleens of fatal cases of botulism.

B. botulinus has been found by several investigators in the dejecta of animals experimentally fed with material containing toxin and spores of this organism, but we have seen no record of the finding of the toxin in the stools of clinical or experimental cases. Meyer and Geiger[17] give in detail their studies of the stools and tissues of various fatal cases of botulism, in man and in cattle. In the former, they obtained both types of the organism from the stools at varying periods after the causative meal was eaten, as well as from the wall of the jejunum in one case and from several parts of the intestine in another, but their attempts to recover the organism from the spleens in three cases were negative. The foregoing positive findings were reported, as well as "isolations from the liver, mesenteric lymph nodes, etc.," of cattle. Records and Vawter,[18] in a study of diseases of cattle report the recovery of both types of B. botulinus from infarcts of the liver.

Recently Tanner and Dack[19] published positive findings of B. botulinus in 2 out of 10 samples of stools from normal persons. Dubovsky and Meyer,[20] in a search for this organism from the stools of 45 normal persons, reported entirely negative results. In view of these results and in considering the reported positive findings from laboratories where constant work of this character is in progress, the possibility of contamination even by experienced workers should always be borne in mind.

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[12] Abstr. Bacteriol., 1920, 4, p. 10.
[13] Jour. Am. Med. Assn., 1918, 71, p. 518.
[14] Ztschr. f. Hyg. u. Infektionskr., 1897, 26, p. 4.
[15] Ztschr. f. Chemotherap., O., 1913, 1, p. 458.
[16] McCaskey: Am. Jour. Med. Sc., 1919, 158 p. 57.
[17] Pub. Health Rept., 1921, 36, p. 1313.
[18] Jour. Am. Med. Assn., 1922, 79, p. 132.
[19] Jour. Am. Vet. Med. Assn., 1921, 60, p. 155.
[20] Jour. Infect. Dis., 1922, 31, p. 501.

Dubovsky and Meyer,[20] in a series of human cases seem to have taken cultures from more of the organs than we have found reported elsewhere—they studied the spleen, liver, brain, lungs, kidneys and wall of the jejunum, ileum and colon, besides the intestinal contents. In 4 cases studied they found B. botulinus, types A and B, in the intestinal contents, and in various parts of the wall itself in cases from the outbreak at Florence, Arizona; and once in the liver and intestines of one case in the Healdsburg outbreak, though the findings for the other organs were negative. Their direct toxin tests from all this material also gave negative results. The same authors also obtained cultures from the organs and intestinal contents of horses, cattle and chickens, as well as from the crops of the latter. While complete findings are not given, they mention having recovered the organism from the spleens and livers of chickens, also frequently from their crops and intestines. The livers and mesenteric lymph nodes from 2 cows were positive. They admit in their article, which embraced work undertaken over a year ago, that it had not been proved that there is an etiologic relationship of these organisms to the lesions or organs from which they have been isolated and comment on the significance of the findings from the crops and intestinal contents of chickens which swallow large quantities of soil. In the light of these findings, Dickson's failure to recover B. botulinus from the colon and ileum of 250 grain fed hogs does not seem remarkable, providing these hogs were not fed other foods which might have been grossly contaminated with spores of B. botulinus. It is possible, however, that these negative results are due to the taking of cultures from insufficient fecal material, as the work of this laboratory along these lines has shown that occasionally these spores may be found in the feces of normal guinea-pigs and rabbits as well as in grain.

The records of the study of the pathology of the various organs in human beings and animals which have shown symptoms of botulism poisoning are even more meager than the reports of cultural studies. A few European workers and Wilbur and Ophüls,[21] Armstrong, Story and Scott,[6] Meyer and Geiger,[17] and Dickson[22] in this country, mention the histologic changes found. Most of these authors noted a characteristic type of thrombus formation in the vessels of the brain. Dickson[23] believes that these thrombotic changes are not responsible for the symptoms of botulism, as they are rarely found in animals which have died within 48 hours after receiving an injection. In a recent important communication, Warthin[24] describes not only the lesions encountered in the brains of 6 human cases studied, but remarks that "all presented similar features of antemortem gas production in the brain and formation of small gas cysts containing bacilli morphologically resembling B. botulinus. These cases present evidence that human botulism is an infection as well as an intoxication."

Some of the negative results in the search for B. botulinus in the organs by the various workers may be due to the cultural methods employed, to the delay in taking cultures or previous chemical treatment of necropsy material from human cases. However, an analysis of the results obtained as well as a consideration of the opinions expressed shows an increasing probability that further study along these lines will definitely decide the question as to the growth of B. botulinus in the animal body.

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[21] Arch. Int. Med., 1914, 14, p. 589.

[22] Monograph, Rockefeller Institute, No. 8, 1918.

[23] Jour. Am. Med. Assn., 1921, 77, p. 483.

[24] Ibid., 1922, 79, p. 71.

TECHNIC

The spores of B. botulinus used were obtained from cultures in veal peptic digest gelatine, from 2 to 4 months old. They were washed from 1 to 3 times in 60 to 75 c c of saline phosphate, suspended in the same medium, heated 1 hour at 80 C. in a sealed tube at the same time as the supernatant culture fluid and final wash water, and injected or fed immediately thereafter. Guinea-pigs were used in our experiments unless otherwise stated. They were usually fed while fasting and invariably with 1 c c of suspension by means of a pipet attached to a rubber tube held in the operator's mouth. As a control for toxicity of the culture from which the spores were obtained and for the detoxification of the spores themselves, both heated and unheated supernatant culture fluid and heated and unheated final wash water were injected subcutaneously into guinea-pigs. This precaution was always taken and, in order to save repetition, the protocols of these injections for each experiment will not be given. Exceptions to this technic are to be noted in the experimental data given in tables 1 and 2.

Direct microscopic spore counts checked by cultivated dilutions in both beef heart and agar were made immediately after heating and the approximate number of vegetative forms noted. The purity of the spores was verified later from the cultivated dilutions.

A rather full consideration of the technic employed in the necropsies and taking of culture from the organs of our animals may not be out of place in view of the negative results obtained by the early workers and the paucity of records of positive findings of the organism in the various organs by recent investigators.

Guinea-Pigs.—It is essential to use animals from a healthy stock in order to avoid unobserved symptoms or death from an intercurrent infection, with consequent distribution of various contaminating organisms throughout the body. As it is possible, where B. botulinus is a common soil anaerobe or when the guinea-pigs have been kept in cages possibly grossly contaminated with B. botulinus, to find this organism occasionally in the feces and rarely in the organs of apparently normal animals, it is imperative that each guinea-pig used should be from strictly fresh stock and placed and maintained separately in small cages which have been adequately sterilized. This we have done.

Necropsy.—Instruments have been sterilized in paraffin oil for 10 minutes at 150 C., cooled and, when not in use, kept covered with a sterile towel throughout the operation. The guinea-pigs have been completely immersed from 1½ to 2 minutes in paraffin oil at the temperature noted, drained for a moment and placed on a thoroughly flamed necropsy board. Sometimes, particularly during necropsy of our controls, a sterile towel also has been laid on the board. Separate instruments have been used in the handling of each organ. The pleural and abdominal cavities have been opened separately to avoid the spread of blood. The assistance of an aide and rapid work greatly minimize the opportunities for contamination. In spite of the most rigorous precautions, some contaminations probably occurred from the air or were originally present in the organs of the animal.

TABLE 1

Results of First Experiments, Performed with Strain 97, Type A — Many Rods

No.	Guinea-Pig Weight, Gm.	Dose	Product	Symptoms	Survival	Positive Organs	Remarks
			Toxic Spore Suspension, Subcutaneous				
1	300	0.1 c c	Supernatant toxin	Botulism	Died after 45 hrs.		
2	300	0.1 c c	Supernatant toxin heated 1 hr. at 80 F.	....	Survived	....	Centrifugalized 1½ hrs.
3	280	1.0 c c	Second wash before heating	Botulism	Died after 21 hrs.	....	Centrifugalized ½ hr.
4	290	1.0 c c	Second wash after heating	Botulism	Died after 5 days	Spleen, mes. liver, blood bone marrow (mice ++)	Centrifugalized ½ hr.
5	280	2.0 c c	Second wash after heating	Botulism	Died after 45 hrs.	....	Centrifugalized ½ hr.
			Spore Injections, Subcutaneous (Dose, 2 c c)				
7	244	1,120 B	....	Botulism	Died after 24 hrs.	Spleen, liver (mice ++)	
8	247	1,120 B	....	Botulism	Died after 20½ hrs.	Blood Spleen Liver } mice + Stomach	
9	279	560 M	....	Botulism	Died after 24 hrs.	Bonemarrow Mes. Liver } mice + Spleen	
10	258	560 M	....	Botulism	Died after 24 hrs.	Liver, spleen blood mouse ++	
12	330	112 M	....	Botulism	Died after 4 days		
13	345	112 M	....	Botulism	Killed after 7 days, moribund	Liver Brain Ing. } mice + Spleen	
14	308	11.2 M	....	Not botulism	Died after 18 days	....	General infection, gained weight
15	395	11.2 M	....	None	Survived		
17	320	1,120 M	....	None	Survived		
18	265	1,120 M	....	None	Survived		
			Fed Spores (Fed While Fasting)				
20	310	2,240 B	....	Botulism 7 days	Killed after 8 days	Blood, spleen, mes., liver	All mouse +
21	410	2,240 B	....	Botulism 7 days	Killed after 8 days	Liver, spleen, mes., R. bone	All mouse +
22	380	1,120 B	....	None, lost weight	Killed after 10 days	Lung, bone, liver	All mouse +
23	320	1,120 B	....	None, lost weight	Killed after 10 days	Mes., bone, spleen, ing., liver	All mouse +
24	250	560 M	....	None, gained weight	Killed after 15 days	Bone, lung, spleen, ing., liver, feces	Feces mouse +
25	250	560 M	....	None, gained weight	Killed after 15 days	Ing., feces	Feces mouse +
26	240	280 M	....	None, gained weight	Killed after 16 days	Inguinal lymphnode	
27	240	280 M	....	None, gained weight	Killed after 16 days	Liver, lungs	
29	250	140 M	....	None, gained weight	Survived		
30	280	140 M	....	None, gained weight	Survived		
31	250	70 M	....	None, gained weight	Survived		
32	250	70 M	....	None, gained weight	Survived		
19	350	Check	....	Lost weight	Died after 19 days	Ing., kidney (mice ++)	Intercurrent infection

Ing., inguinal lymphnode; Mes., mesenteric lymphnode; Bone, bonemarrow; B, billion; M, million.

It is to be borne in mind that the subsequent use of the word contaminated as applied to the culture of organs does not necessarily imply contaminations from without. In cases in which organisms so closely associated culturally and morphologically as B. botulinus and B. sporogenes, pathogenic and slightly pathogenic, are growing together in the intestinal tract, it is not unreasonable to suppose that the invasion of the organs by the former may carry through certain individuals of the other species. Heller[25] mentions that while B. sporogenes is not invasive in small doses in pure culture, it may invade the tissues in company with other organisms. This appears to be true for other anaerobes as well. Also since it has been recently stated[26] that the gallbladder and its ducts in 63% of human cases brought to operation contained bacteria, it is not surprising occasionally to find invasive organisms other than B. botulinus in the organs of our guinea-pigs. Stress is laid on these facts in order that the thoroughness of our technic may not be questioned.

Cultures.—The beef heart and liver agar mediums and the reasons for their use in the botulism work of this laboratory have already been fully discussed by Dubovsky and Meyer.[20] As the final determination of the presence of B. botulinus in cultures, unless antitoxin neutralization tests are to be made, depends on the heat resistance of its spores, we have found it desirable to use this medium.

The organs were placed in the freshly boiled and cooled beef heart medium and incubated from 7 to 15 days at 35 C. and reincubated when necessary. All cultures, regardless of appearance or lack of odor, were examined microscopically, and material from those tubes which showed sporulating organisms in any way morphologically resembling B. botulinus or free spores with or without bacilli, were inoculated into deep agar, heated from 1 to 1½ minutes at 100 C., incubated from 1 to 7 days at 35 C., and the character of the colonies observed. When such colonies or a sufficient number of them were doubtfully positive for B. botulinus, the original beef heart culture was inoculated into mice. In many apparenlty sterile beef heart cultures a long search will result in the finding of an occasional free spore where nothing else is seen. These spores may or may not prove to be viable. In contaminated cultures, more particularly in those in which the contaminating organisms are cocci or anaerobes of the B. bifermentans or B. sporogenes types, the latter may almost completely overgrow the few organisms or spores of B. botulinus originally in the culture, as has been explained by Reddish.[27] In this paper, he also mentions the possibility of an agar colony of B. sporogenes containing within its body spores of B. botulinus. The impossibility of seeing these, as well as the failure to recognize the atypical colonies of B. botulinus, may have caused us to give some erroneously negative findings. Considering the foregoing as well as other sources of error, and in spite of the precautions taken, we believe that we have sometimes failed to show the presence of B. botulinus spores in the organs of our experimental animals and that the results given in our tables are possibly too low. However, we wish to state that in the organs of our controls every possible method was used to demonstrate the presence of the organism.

Cultures of the following organs were always made: Blood, bone marrow, inguinal and mesenteric lymph nodes, liver and spleen, and often the brain, lungs, kidneys and sometimes the feces and thigh muscle and gallbladder. The results of our experiments are now presented.

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[25] Jour. Bacteriol., Jan. 1922, 7, p. 29.

[26] Beitr. z. klin. Chirurg., 1922, 125, p. 377.

[27] Jour. Infect. Dis., 1921, 29, p. 126.

EXPERIMENTAL DATA

Our preliminary experiments (tables 1 and 2) do not conform in all respects to the description of our technic, but as the results have an important bearing on the subject to be discussed later, we have decided to include them. The spore suspension in the first experiment and the heated and unheated toxin and final wash water (table 1) were ready too late on a Saturday afternoon to inject and so were left in the icebox until the following Monday morning (about 40 hours), when they were injected. They proved to be toxic.

TABLE 2

RESULTS OF EXPERIMENTS WITH STRAIN 38, TYPE A—FEW VEGETATIVE FORMS

Toxic Spore Emulsion; Final Heated Wash Water (Icebox 48 Hrs.) Subcutaneous

No.	Guinea-Pig Weight, Gm.	Dose	Symptoms	Survival	Positive Organs	Remarks
52	178	2 c c	Botulism	Died after 9½ days	All but brain	
53	170	2 c c	Botulism	Died after 4 days	Liver, spleen	
54	158	1 c c	Botulism	Died after 12 days		
55	150	1 c c	Botulism	Died after 11 days		
				Subcutaneous Spore Injections		
56	294	1.8 B	Botulism	Died after 20 hrs.	All + but ing. and brain	
57	248	1.8 B	Botulism	Died after 5½ days	Brain, liver, bone, mes., blood	
58	255	900 M	Died in night	Died after 18 hrs.	Ing., blood, mes., spleen bone	
59	240	900 M	Botulism	Killed after 3 days	Mes., spleen, liver, bone	
60	199	180 M	Botulism	Died after 14 days		
61	222	180 M	None	Survived		
62	230	Check	None	Killed after 16 days	Bone (mouse +) all other organs negative	
63	205	18 M	Indefinite	Died after 12 days	....	Intercurrent infection
64	202	18 M	Indefinite	Died after 13 days	....	Intercurrent infection
65	217	1.8 M	Indefinite	Died after 7 days	....	Intercurrent infection
66	203	1.8 M	Indefinite	Survived		
				Fed Spores (Fed While Fasting)		
67	220	3.3 B	Botulism	Died after 4½ days	Ing., liver, stomach	
68	300	3.3 B	None	Killed after 10 days	Ing., liver	
69	200	1.650 B	None	Survived		
70	190	1.650 B	None	Survived		
72	165	1.250 B	None	Survived		
73	183	1.250 B	None	Survived		
74	181	725 M	None	Survived		
75	222	725 M	None	Survived		
	240	Check	None	Killed after 16 days	All organs negative	

As these results were so at variance with those of previous workers, even though the wash water containing few spores and consequently the spore suspension (afterward diluted) were toxic, we decided to repeat this experiment under similar conditions with a portion of the emulsion used in the experiment summarized in table 3.

In this duplicated experiment, the heated spore suspension and final heated wash water were left in the icebox for 48 hours (Table 2).

These two experiments show the necessity of injecting spore suspensions immediately after heating.

The result of our next experiment, in which the spores were completely detoxified, is given in table 3.

As will be seen from a comparison of the data given in the foregoing tables, an enormous number of B. botulinus spores are necessary to produce symptoms and death, especially when fed to guinea-pigs.

As we were able to cause symptoms or death in our guinea-pigs only by the injection and feeding of spores of B. botulinus in doses considerably in excess of those employed by other investigators, and as we found a similar irregularity in our results, we thought it would be of interest to ascertain whether it were possible to break the apparent immunity of some of our animals to large doses. Several of these guinea-pigs had survived without presenting symptoms, while others receiving the same dose succumbed.

It has long been known that the spores of pathogenic bacteria, when freed from their toxin, may remain latent in the tissues of the body for considerable periods, and when anything happens, such as slight localized infections or prophylactic vaccinations, to upset the equilibrium of the bodily defenses or to break down its resistance, such spores may germinate and cause infection. An intensive study of this question has been undertaken by different investigators.

Koser and McClelland[28] have shown that while it is unusual to recover the spores of the aerobes away from the site of inoculation, the spores of the anaerobes may be recovered from the different organs up to 4 months after inoculation. They confirm the results of many others, especially as regards B. tetani, which has been found in most of the organs of the body in fatal human cases, as well as in experimental animals. Canfora[29] recovered free spores of B. tetani from the blood stream as early as 12 hours and up to the seventieth day after subcutaneous injection. Vaillard and Vincent,[30] working with lactic acid and B. prodigiosus, Massart and Bordet,[31] with lactic acid,

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[28] Jour. Med. Res., 1917, 37, p. 259.

[29] Centralbl. f. Bacteriol., I, O., 1908, 45, 495.

[30] Ann. de l'Inst. Pasteur, 1891, 5, p. 1.

[31] Ibid., 1891, 5, p. 417.

Semple,[32] with quinine, and Teale and Bach,[33] with lactic acid, sodium bicarbonate, calcium chloride and other substances, have all been able, under varying conditions, to break down the resistance of the host to the spores of B. tetani or of other bacteria by the injection of these substances. The latter workers believe from their experiments that, while sometimes the spores do not germinate, at other times they do, but that they do not appear to multiply in the tissues. In many of the different experiments cited in the foregoing, cultures of the organs gave positive findings for the spores of the species of organism injected. Tulloch[34] injected 1,000,000,000 toxin-free spores

TABLE 3

RESULTS OF EXPERIMENTS WITH STRAIN 38, TYPE A — FEW VEGETATIVE FORMS

Nontoxic Spore Emulsions; Injected Immediately After Heating

No.	Guinea-Pig Weight, Gm.	Dose	Product	Symptoms	Survival	Positive Organs	Remarks
34	410	0.1 c c	Supernatant toxin	Botulism	Died after 24 hrs.		
35	360	1.0 c c	Supernatant toxin 1 hr. at 80 C.	None	Survived		
36	357	1.0 c c	Second water before heating	Botulism	Died after 24 hrs.		
37	245	1.0 c c	Second water after heating	None	Survived		
39	300	1.0 c c	Second water after heating	None	Survived		
40	278	1.0 c c	Second water after heating	None	Died after 14 days	....	Intercurrent infection
41	270	2.0 c c	Second water after heating	None	Survived		
42	270	2.0 c c	Second water after heating	None	Survived		
43	250	2.0 c c	Second water after heating	None	Survived		
			Fed Spores (Fed While Fasting)				
45	275	11 B	....	Botulism 60 hrs.	Died after 96 hrs.	All + except ing.	Brain not cultured
46	272	11 B	....	Botulism 22 hrs.	Died after 60 hrs.	All +	Lung, kidney not cultured
47	242	5.5 B	....	Botulism 60 hrs.	Killed after 71 hrs., moribund	Liver, kidney, bone, brain, blood, mes.	
48	210	2.75 B	....	None	Survived		
49	229	5.5 B	....	None	Killed after 14 days	Ing.	
50	192	2.75 B	....	None, lost 40 gm.	Died after 14 days	All + except lungs	
C	350	Check	....	None	Survived		

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[32] Scient. Mem. Officers and San. Dept. Gov't. India, 1911, No. 43, new series (cited by Koser and McClelland).

[33] Jour. Path., 1920, 23, p. 315.

[34] Brit. Med. Jour., 1918, 1, p. 614.

of B. tetani into animals without producing the corresponding disease. The subsequent inoculation of B. welchii toxin caused the germination of the spores of B. tetani. The experimental data summarized in tables 1 and 2 tend to confirm to a certain extent the work of Tulloch as to the accessory action of a toxin, in this case homologous, more especially when the toxic spores were subcutaneously administered. As we were able to produce botulism by feeding toxic spores only in excess of 1,120,000,000 in one case and in excess of 1,650,000,000 in another, it is possible that, due to various factors, absorption of a slight amount of toxin from the intestinal tract is prevented. This phase of the question will be referred to later. When the results of these tables are compared with those recording our experiments with nontoxic spores (table 3) and those to be given later, the differences between our results, especially in subcutaneous injections with toxic and toxin-free spores and those of other workers is apparent. The observations of Geiger, Dickson and Meyer[10] as to the difficulty of detoxifying spores by washing or slight heating at 80 C., as many of these investigators have done, are borne out, and this may be one of the causes of the discrepancies between their results and ours. The cultural findings in guinea-pig 52 (table 2) which received toxic wash water subcutaneously is interesting. While the injection of this water would probably have caused a fatal outcome in this case had it not contained very few spores, its toxicity would appear to have been instrumental in causing the spread of these spores, or the bacilli possibly arising from them, throughout all the organs of the body except the brain. This does not seem to occur even in subcutaneous injection unless many millions of detoxified spores are introduced. Here, again, we probably have a confirmation of Tulloch's findings.

Bullock and Cramer[6] and Edmondson, Giltner and Thom[5] have shown that calcium chloride injected at the same time or within a few minutes after the subcutaneous injection of pathogenic spores, the latter working with B. botulinus, greatly diminishes the time necessary to produce symptoms or death. The latter workers showed that such injections produced no effect if given two days after the spore injection. Their animals were all injected subcutaneously both times.

Though Teale and Bach[33] had fewer failures by the intraperitoneal route, our injections were generally made subcutaneously within 1 to 2 cm. of the site of the original injection. Proper controls for each substance were also made. In view of the results of the latter workers, we are not altogether surprised at our failure by this means, after the interval shown in our tables, to alter the effect of the spores when either fed or injected. Our cultural studies of the organs of other animals lead us to suppose that the tissues of the guinea-pigs thus injected had already been invaded by the spores or bacilli at the time of the injection of such additional substances, and any negative chemotactic property these substances may have possessed was useless at the time they were injected, so that this failure must be attributed to a different cause. Tables 4, 5 and 6 show our experiments in detail.

During the course of these experiments, we endeavored to determine how rapidly the spores of B. botulinus leave the site of subcutaneous and intraperitoneal injections. We inoculated 2 c c of a suspension of detoxified spores containing a few bacilli, some of which colored partially by the Gram method, into the peritoneum of 2 guinea-pigs, one of which had been previously injected in the same manner with 5 c c of sterile broth. At 15 minute intervals after injection of the spores, a few drops of fluid were withdrawn from each animal and stained by the Gram method on a slide containing a smear from the spore suspension inoculated. No microscopic differences were apparent in preparations from the 2 guinea-pigs. The leukocytes englobed the

TABLE 4

RESULTS OF EXPERIMENTS WITH STRAIN 58, TYPE A — VERY FEW RODS

Subcutaneous Injections

No.	Guinea-Pig Weight, Gm.	Spores	Symptoms	Survival	Organs Positive	Remarks
80	269	504 M	Botulism	Died after 15 days	All organs + spores in liver	
81	275	504 M	None	Killed after 25 days	Spleen, liver, mes., ing.	After 9 days received 0.5 cc N. Na_2CO_3 intraperitoneally, no symptoms
82	254	252 M	None	Died after 15 days	All organs + spores in liver	
83	227	252 M	None	Killed after 21 days	Liver, bone, ing.	After 9 days received 0.5 cc N. Na_2CO_3 intraperitoneally, no symptoms
84	225	42 M	None	Killed after 24 days	Liver, spleen, kidney, bone, mes.	After 8 days received same as above
85	214	42 M	None	Survived	....	After 14 days received 0.5 c c N. lactic acid intraperitoneally, no symptoms
86	214	Check	None	Survived		
Fed Spores (Fed While Fasting)						
87	223	Check	None	Survived	....	Necropsy, table 6
88	300	2.520 B	None	Killed after 10 days, gained 60 gm.	Liver, brain, L. bone	
89	296	2.520 B	Typical botulism 7 days	Killed after 8 days, moribund	Lungs	
90	288	1.008 B	None	Killed after 24 days	Ing., feces	
91	280	1.008 B	None	Survived	....	After 13 days fed 0.00675 gm. quinin sulphate, no symptoms
92	259	504 M	None	Survived		
93	258	504 M	None	Killed after 27 days, gained 118 gm.	Bone, feces +	
94	251	252 M	None	Killed after 24 days, gained 92 gm.	All negative, feces +	
95	248	252 M	None	Survived	....	After 9 days received 0.5 cc N. lactic acid intraperitoneally, no symptoms
96	243	Check	None	Survived	All negative	
97	242	1.176 B	None	Killed after 21 days	Liver	After 14 days received 0.005 gm. quinin sulphate subcutaneously, no symptoms

spores and bacilli, and at the end of 6 hours they had practically disappeared from the site of inoculation. Three guinea-pigs were injected subcutaneously with 2 c c of an emulsion of detoxified spores, a pocket having been made under the skin with the needle of the syringe. One guinea-pig also received mixed with the spores, 1 c c of a 2% solution of calcium chloride and another. 1 c c of B. botulinus antitoxin added to the spores. The death of the guinea-pig receiving calcium chloride preceded that of the control by a long period of time. Again no differences were noted in the stained preparations after varying intervals up to 24 hours. In the beginning, few leukocytes were present, but they gradually appeared until at the end of 24 hours an intense leukocytosis was present. The disappearance of the spores and bacilli was rapid, and at the end of 5¾ hours few were microscopically visible, either free or in the leukocytes. A few rare leukocytes at this time were gorged with spores and bacilli both in the guinea-pig receiving antitoxin and in the one receiving calcium chloride. Saline washings from these pockets were nontoxic for mice. While it is more than likely that some of the injected spores germinated in these pockets or in the peritoneum within 24 hours, we have no reliable evidence that this was the case. There were not sufficient differences between the Gram stained preparations from the animal fluids and the original spore material on the same slide to warrant a definite statement that this is so. We have sometimes noted a few gram-positive organisms in our heated spore suspension, so that unless there is marked growth or additional evidence is obtained that these rare bacilli seen within a short time after injection are B. botulinus, one should accept with reserve these findings as evidence of growth. The result with calcium chloride is not in accord with that of Thom, Edmondson and Giltner,[5] who found that the leukocytes did not englobe the bacilli and presumably also the spores of B. botulinus when injected with this substance. These authors remark that smears showed a few bacilli in the exudate from these subcutaneously injected guinea-pigs and that "cultures revealed the presence of the bacilli in virulent form," but they do not state how they determined that their cultures originated from bacilli rather than from spores. They suggest from the presence of bacilli on their smears that growth of B. botulinus must have occurred in the animal body, but they do not mention any controls of colorations of the spore material injected, nor do they state the method used to color their smears nor describe the bacilli seen. Their results are interesting but need amplification.

Teale and Bach[33] have stated that they failed to prevent the phagocytosis of bacteria by injecting lactic acid simultaneously with the bacteria. These authors discuss at length the question of latency of anaerobic organisms and believe that the spores germinate and the vegetative forms thus arising remain latent. Whatever may be the mode of action of these accessory factors in accelerating the appearance of B. botulinus symptoms such action would not seem to be due entirely to any negative chemotactic property which such substances may possess for the leukocytes or other phagocytes.

Several of the authors previously quoted have considered that the pathogenicity of the detoxified spores and killed vegetative forms might be due to the action of the enzyme of the blood or tissues in liberating whatever preformed toxin they might contain. While this possibility, for various reasons, seems to us exceedingly remote, we thought it worth while to try the following experiment: Emulsions of counted spores of 2 strains and 1 other in which the number of spores was unknown were heated in sealed tubes at 122 to 125 C. for 5 minutes. The tube in which the spores were uncounted

TABLE 5

RESULTS OF EXPERIMENTS WITH STRAIN 97, TYPE A — MODERATE NUMBER RODS; FEEDING EXPERIMENTS

Guinea-Pigs

No.	Weight	Product	Symptoms	Survival	Positive Organs	Remarks
98	450	5.08 B + ½ c c N. serum subcutaneously	None, lost 66 gm.	Killed after 30 days	Lungs, brain, feces	After 10 days received 0.005 gm. quinin sulphate subcutaneously, no symptoms
99	420	5.08 B + 1 c c antitoxin A subcutaneously	None, gained 10 gm.	Killed after 30 days	Lungs	
100	410	5.08 B + 1 c c N. serum subcutaneously	Not botulism, lost 120 gm.	Died after 5 days	Liver	Paratyphoid
101	354	5.08 B + 1 c c antitoxin A subcutaneously	Not botulism, lost 29 gm.	Died after 3 days	Brain, liver, L. bone	General infection
102	259	Check	Not botulism	Died after 13 days	All organs negative	

Mice

1	Medium size	1.27 B + ½ c c N. horse serum subcutaneously	None	Killed after 10 days	No cultures	
2	Medium size	1.27 B + ½ c c antitoxin A subcutaneously	None	Killed after 10 days	Liver, spleen, bone	
3	Medium size	1.27 B + ½ c c N. serum subcutaneously	None	Killed after 10 days	Blood, bone	
4	Medium size	1.27 B + ½ c c antitoxin A subcutaneously	Indefinite	Died after 2½ days	Liver, spleen	
5	Medium size	Check	....	Killed after 10 days	All negative	

Rabbits

	2,600	63.5 B	None until 31st day dysentery, gained 200 gm. to 20th day	Killed after 35 days	Bone, ing., feces	After 31 days, fed 25,000 M.L.D. filtered toxin; no symptoms except continuation of dysentery

and which contained enormous numbers was immediately opened, 3 drops inoculated into beef heart and the remainder (about 2 c c) injected into a guinea-pig. The other 2 tubes, which contained 17,000,000,000 and 20,000,000,000 spores, respectively, were placed in the incubator and icebox alternately for several hours during 6 days. They were then opened, inoculations made into beef heart and the remaining contents centrifugalized. The deposit and supernatant fluid from each tube (about 1.5 c c) were injected separately under the skin of different guinea-pigs. None of these injections produced symptoms, showing that the protein substance, or its autolyzed products of killed B. botulinus spores and rare bacilli in the amounts injected is not toxic when introduced subcutaneously into guinea-pigs. The inoculated mediums from all these suspensions remained sterile.

TABLE 6

RESULTS OF EXPERIMENTS WITH STRAIN 62, TYPE A — RARE VEGETATING FORMS

Subcutaneous Injections

No.	Weight	Dose	Symptoms	Survival	Organs Positive	Remarks
109	252	1.459 B	Botulism	Killed after 4 days, moribund	Mes., liver	
110	250	1.459 B	Botulism 24 hrs.	Died after 43 hrs.	All organs +	
111	247	230 M	None	Killed after 20 days	Liver, spleen, ing., bone, mes.	
112	232	230 M	None	Killed after 20 days	Ing. (mouse +)	After 23½ hrs. received 0.9 c c 2% N. $CaCl_2$ subcutaneously, no symptoms
113	207	230 M	None, 21 days gained 45 gm.	Survived		
114	200	230 M	Botulism	Died after 4½ days	Ing., bone, spleen, liver, brain, blood	After 23½ hrs. received 0.9 c c 2% N. $CaCl_2$ subcutaneously, no symptoms
115	285	Check	None	Survived		
			Fed Spores (Fed While Fasting)			
117	320	11.5 B	Botulism 3 days	Killed after 4½ days moribund	Bone, brain, liver, feces	Lost 73 gm.
118	289	11.5 B	Indefinite	Died after 3½ days	Mes.	Lost 29 gm.
119	286	5.75 B	Botulism 6 days	Died after 13 days	Liver, feces	Lost 126 gm.
120	282	5.75 B	Botulism	Died after 6½ days	Liver, bone, brain	Lost 100 gm.
121	270	2.875 B	None	Survived	....	Released after 5 wks.
122	270	2.875 B	None	Killed after 15 days	Lungs, brain, feces	Gained 40 gm.
123	260	1.150 B	None	Killed after 15 days	All negative	Gained 52 gm.
124	258	1.150 B	None	Killed after 15 days	All negative	After 22¼ hrs. received 0.9 c c 2% N. $CaCl_2$ subcutaneously, no symptoms, gained 79 gm.
125	177	1.150 B	Indefinite	Died after 4 days	Liver, feces	Lost 14 gm.
126	200	1.150 B	Lost 67 gm.	Died after 16 days	All negative, feces +	After 22¼ hrs. received 0.9 c c 2% N. $CaCl_2$ subcutaneously, no symptoms
87	330	Check	None	Killed after 16 days	Liver, feces (mice ++)	Gained 50 gm.

Though the wall of a viable spore may be highly resistant to heat, we know that when subjected to a temperature of 80 C. for 1 hour the protein contents undergo some damaging changes, if not a certain degree of coagulation, as often evidenced by greatly delayed ability to germinate after being heated at this temperature. Also, it probably could be shown by quantitative cultural studies before and after heating to 80 C., as has been done for a temperature of 100 C., that many of the least resistant spores had been killed at this temperature. That is why we have always checked our microscopic counts by cultivated dilutions after heating. Since the temperature at 80 C. produces some of these vital changes, it is more than likely that it destroys the intracellular toxin of the spores as well.

A summary of the toxicity of the different strains employed in the foregoing experiments, as well as the minimum dose required to produce definite symptoms of botulism is given in table 7.

TABLE 7

Toxicity of Strains

Strain	Toxic Spore Emulsion	Minimum Dose Given Subcutaneously	Death from Botulism	Minimum Dose Fed	Death from Botulism	Remarks
97	Yes	112 M	4 and 7 days	2.24 B	Killed after 8 days	Very ill; rats killed
97	No	......	....	5.08 B	2 survived	1 received antitoxin
97	No	......	....	5.08 B	....	2 died, after 3 and 5 days, botulism very doubtful; 1 received antitoxin
97	No	......	....	63.5 B	(Rabbit) survived	Killed after 35 days
38	No	......	....	2.75 B	14 days, 1 survived	
38	Yes	180 M	14 days, 1 survived	3.3 B	4½ days, 1 survived	
58	No	252 M	15 days, 1 survived	2.5 B	7 days, 1 survived	
62	No	280 M	4½ days, 3 survived	5.75 B	13 days, 6½ days	

From table 7 it will be seen that strain 58 was the most toxic in the feeding experiments, while strain 38 appears to be the most toxic of those injected subcutaneously. However, due to the few experiments recorded with each strain, the individual susceptibility of the animals or other unknown causes, no definite conclusions can be drawn as to any differences which may exist in the pathogenicity of the spores of each strain. Owing to the fact that many of our guinea-pigs were killed with a view to the study of the organs, perhaps some of these might have developed symptoms had they been allowed to live longer. For this reason, we do not categorically deny the possibility of producing botulism for these strains in doses below those given in table 7, but it would appear that these strains, in our hands, all of which produced culturally a potent toxin, were not pathogenic in small doses like those recorded by other workers for other strains.

A summary of the cultural studies of the various organs of our experimental animals, as shown in the tabulation given in the foregoing, is given in tables 8 and 9. In figuring the percentages of the results of this work, we have included cultures of the organs from a few animals (intraperitoneal and subcutaneous leukocyte tests) that received detoxified spores but which are not included in our tabulated results.

While, as we have said before, we do not believe that we have been successful, especially in our earlier experiments, in demonstrating B. botulinus in all of the organs when it has been present, the main fact to be deduced from these results is the invasion of all the organs of the body regardless of the mode of administration of the spores. In those animals in which a subcutaneous injection of spores produced a fatal result, the organism was recovered more often than in those that died after the spores were fed. In the foregoing experiments the few microscopic examinations of smears of these organs, or of the heart blood, which we have made have been negative, with the exception of a few cases in which we have seen rare spores or bacilli in the liver and blood. Undoubtedly enormous numbers of the spores are eliminated by the leukocytes of the blood or by the endothelial cells of the tissues, especially of the liver and spleen.

TABLE 8

RESULTS OF CULTURAL STUDIES OF VARIOUS ORGANS OF EXPERIMENTAL ANIMALS

Organ	Subcutaneous						Fed						Total + Died or Dying of Botulism
	Died or Dying		% +	Killed, No Symptoms		% +	Died or Dying		% +	Killed, No Symptoms		% +	
	+	—		+	—		+	—		+	—		
Ing. lymph	5	12	30	3	3	50	4	14	22	4	17	19	25%
Bone	11	6	64	2	4	33	8	10	44	8	13	40	54%
Blood (heart)	9	8	53	0	6	0	2	16	10	0	21	0	31%
Mes. lymph	10	7	59	2	4	33	7	11	40	1	20	5	50%
Spleen	13	4	77	2	4	33	6	12	33	3	18	14	54%
Liver	16	1	94	3	3	50	14	4	80	8	13	38	86%
Kidney	5	1	83*	1	0	—	4	9	32*	0	9	0	48%*
Brain	6	2	75*	0	4	0	7	6	54*	3	14	12	60%*
Lungs	2	1	66*	1	1	—	3	5	37*	8	9	47	45%*
Muscle	0	1	0	0	0	0	0	2	0	0	1	0	0
Feces	0	0	0	0	0	0	3	0	100*	11	2	91*	100%*
Stomach†													
Total guinea-pigs	17			6			18			21			62

* Percentage of times cultures were taken from organ.

† Intraperitoneal 2 guinea-pigs, all + except blood.

To what extent the spores and bacilli of B. botulinus are actually destroyed and where is problematical. The literature on this subject is filled with interesting discussions based on intravenous injections into animals of inert substances and bacteria. Drinker and Shaw[35] Fenn,[36] Nagao[37] and numerous others working with manganese dioxide, carbon, India ink, etc., and Nagao[38] using streptococci, as well as Meyer, Neilson and Feusier,[39] who injected typhoid bacilli, have made a thorough investigation into the fate of these organisms when introduced into the blood stream. The work of the latter authors especially shows the important rôle played by the cells of the lungs, liver, spleen, and bonemarrow and the possibility of reinfection of the blood from foci in these organs. It is possible that the irregular findings

TABLE 9

Animals Killed After Spore Injections

Subcutaneous, No Symptoms nor Lost Weight

Days	Liver	Spleen	Bone	Mes.	Ing.	Brain	Kidney	Lungs	Feces
20	+	+	+	+	+	..	..	..	..
20	..	..	..	..	+	..	..	..	..
21	+	..	+	..	+	..	..	..	..
24	+	+	+	+	..	..	+	..	..
25	+	+	..	+	+	..	..	..	..
	4	3	3	3	4	0	1	0	

Fed, No Symptoms Nor Lost Weight

Days	Liver	Spleen	Bone	Mes.	Ing.	Brain	Kidney	Lungs	Feces
10	+	..	..	..	+	..	..	..	0
10	+	..	+	..	..	+	..	..	..
10	+	+	+	..	..	..	..	..	0 Mice
10	..	..	+	..	..	..	..	..	0 Mice
14	..	..	..	..	+	..	..	..	0
15	+	+	+	..	..	..	..	+	+
15	..	..	..	..	+	..	..	..	+
15	..	..	..	..	..	+	..	+	+
15	0	0	0	0	0	0	0	0	+
15	0	0	0	0	0	0	0	0	+
16	+	..	..	..	..	..	..	+	0
16	+	..	..	..	..	..	..	..	+
21	+	..	..	..	..	..	..	..	0
24	..	..	..	..	+	..	..	..	+
24	0	0	0	0	0	0	0	0	+
27	..	..	+	..	..	..	..	..	+
30	..	..	..	..	..	+	..	+	+
30	..	..	..	..	..	..	..	+	..
35	..	..	+	..	+	..	..	..	+ Rabbit
	7	2	6	0	5	3	0	5	11

No Symptoms, Lost Weight

Days	Liver	Spleen	Bone	Mes.	Ing.	Brain	Kidney	Lungs	Feces
10	+	..	+	..	..	..	..	+	0
10	+	+	+	+	+	..	..	..	0

0 = cultures not taken.

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[35] Jour. Exper. Med., 1921, 33, p. 77.
[36] Jour. Gen. Physiol., 1921, 3, p. 465.
[37] Jour. Infect. Dis., 1920, 27, p. 527.
[38] Ibid., p. 327.
[39] Ibid., 1921, 28, p. 408.

in the heart blood of our experimental animals may be due to this cause. Barlett and Ozaki,[40] who have studied phagocytosis in vivo have shown that the cells of the liver and spleen act to a greater or less extent in a compensatory way for the deficiency of the leukocytes of the blood as they become less capable of ingesting bacteria. They believe this fact to be of great significance in eliminating bacteria from the blood. The fact that we have more often found B. Botulinus in these two organs than in the blood in fatal cases might in a measure corroborate this idea. The same result is also obtained in those cases in which latency of the spores occurs or at least in which symptoms from toxin formation are greatly delayed.

It is unfortunate that the brain was studied in only about 50% of our animals, as the findings for that organ cannot be fairly compared with those of a majority of the other organs. However, from the data at hand, the brain tissue in fatal cases seems to play a rôle second in importance only to that of the liver, or perhaps the spores or bacilli find conditions there more favorable for growth than in many of the other organs. The cultures of the bonemarrow and spleen appear to give equal results for all animals dying of botulism, though in the case of fed animals which presented no symptoms, the organism was found more frequently in the bonemarrow than in any other tissue except the lungs. Allowance must be made in the case of the latter organ for possible direct and continuous contamination from feces and food. The persistence of the spores in the feces of our fed animals which survive long periods is interesting. We have seen no evidence which would support the conclusion that toxin is formed in the intestinal tract in quantities sufficient to cause symptoms of botulism when, or if, absorbed into the blood stream.

Though several slightly pathogenic anaerobes of the putrefactive type as well as some of the highly pathogenic saccharolytic anaerobes, notably B. welchii and B. tetani, lead a semisaprophytic existence in the intestinal tract, the result of the work of this laboratory shows that the spores of B. botulinus are rarely found in the feces of normal animals and have not yet been found in the stools of healthy human beings; so that the possibility that the few spores thus encountered or even greatly increased numbers might produce toxin in the intestines is extremely unlikely. Reference to the data presented in tables 1 and 2 will show the enormous doses of spores even when fed in toxic solution that may be required to produce symptoms of botulism as against the number injected subcutaneously which are required to obtain the same results. This would tend to support our contention. It is another matter when toxin-free spores are accidentally or experimentally ingested in prodigious numbers. They are then frequently introduced into an empty stomach in a concentrated mass and possibly are absorbed from points in the tract where they remain concentrated in the fluid and before any great dissemination throughout the tract has taken place. This is possibly a condition which facilitates their absorption, or at least they penetrate the intestinal wall in numbers too great to be destroyed by the natural defenses of the tissues, where the toxin is probably formed or liberated. Bronfenbrenner and Schlesinger[41] theorize as to points in the digestive tract from which the toxin of B. botulinus is probably absorbed. They have shown that the potency of the toxin is enormously increased in an acid medium and that in addition to its specificity it contains some substance capable of increasing the permeability of the intestine so as to permit the absorption of the specific toxin. Therefore, they believe that when it encounters the acid medium of the upper digestive tract (stomach and upper duodenum), it is readily absorbed. If that be so, it would seem that any toxin which passes this point or is formed in the lower tract might be neutralized, attenuated, or destroyed by the enzymes or alkaline metabolites produced by other bacteria, and that may be why such enormous numbers of detoxified spores, if they produce toxin at all, are innocuous when ingested.

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[40] Jour. Med. Res., 1917-18, 37, p. 139.

Having found B. botulinus so persistently in the organs of our animals when fed or injected subcutaneously with the spores of this organism, we desired to ascertain if possible which organs were invaded first after the spores reached the blood stream. We, therefore, injected the spores intravenously as well as by other routes into a series of animals and killed them at varying intervals. Unfortunately the dose injected intravenously was so large that our results enumerated in table 10 enable us to form no definite conclusions on this subject.

It is evident that a comparatively small number of spores of B. botulinus fed to guinea-pigs do not invade any of the tissues, but the principal information gathered from this experiment proves that the same number of spores, when once in the blood stream, rapidly invade all the tissues of the body. We wish also to call attention to the fact that all the organs of 4 guinea-pigs in this series (2 cage checks and 2 fed) were negative for B. botulinus. This, taken in conjunction with the cultural studies of the organs of our other check animals, in which we have rarely found B. botulinus, should be taken as sufficient evidence that our cultural findings in these experiments are the result of our inoculations of spore suspensions.

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[41] Jour. Am. Med. Assn., 1922, 78, p. 1519.

Meyer, Neilson and Feusier[39] have shown that the distribution and destruction of small or large doses of typhoid bacilli are practically the same in normal and immunized rabbits; so that the question arose in our minds as to whether the neutralization of the toxin of B. botulinus as formed in these intravenous injections could have any influence on the distribution of this organism in the various tissues. We, therefore, inoculated 4 guinea-pigs with a similar dose of a different strain and 10 minutes later injected subcutaneously 2 of them with normal horse serum and 2 with antitoxin corresponding to the type of organism injected. We also endeavored to determine whether there was circulating toxin in the emulsified organs of any of these guinea-pigs, and, finally, whether the cultures of these organs were produced by spores

TABLE 10

RESULTS OF EXPERIMENTS WITH STRAINS 97, TYPE A AND 108, TYPE B MIXED CULTURE; PRACTICALLY NO RODS

No.	Weight	Spores	Symptoms	Survival, Hours	Killed or Died	Positive Organs	Remarks
				Jugular Vein			
131	336	Check	None	114	Killed	None	
132	350	20 M	None	18	Killed	All	
133	360	20 M	None	43	Killed	All	
134	344	20 M	None	46	Killed	All	
135	350	20 M	Botulism	67	Killed, very ill	All	
136	350	20 M	Botulism	70	Killed, very ill	All	
137	323	20 M	Botulism	60	Died	All	
138	356	20 M	Botulism	80	Died	All	
140	256	20 M	None	17	Killed	All except blood, brain, kidney, mes.	Blood coagulated, little taken
141	265	20 M	Botulism	69	Killed, very ill	All	
				Subcutaneous			
142	30[illegible]	20 M	None	85	Killed	Liver, spleen, ing.	
143	315	20 M	None	87	Killed	All	
				Fed			
144	360	750 M	None	Survived			
145	265	20 M	None	95	Killed	None	
147	230	20 M	None	96	Killed	None	
146	200	Check	None	97	Killed	None	

(and bacilli) or by bacilli alone. We found that the emulsified brain, spleen and liver of one guinea-pig (155) injected with antitoxin were toxic for mice in 5, 4 and 6 days, respectively, but the same organs of guinea-pig No. 153, receiving no antitoxin, were not toxic for mice. This result is difficult to interpret, and the experiment should be repeated on a larger scale before forming any conclusions. A microscopic study of the emulsions of the organs gave the following results: Rare spores and gram-positive bacilli were seen in preparations of the blood of No. 152, while the blood of No. 154 was negative. The spleen and liver of No. 153 showed rare gram-positive bacilli, resembling B. botulinus but no spores, while the same organs of guinea-pig 155 were negative. The blood of these last two animals was not examined microscopically. The other results obtained in this series are shown in table 11.

A study of this table shows, as in our previous experiment with intravenous injections, the rapid dissemination of the organism throughout the body. The failure to recover from the brain in most of these cultures is unusual. It is possible that this organ is the last to be invaded, and under the conditions of this experiment the animals died or were killed (except 155) before the organism had lodged in the brain. In experiments previously reported, we have frequently failed to recover the organism from the brains of animals dying within 48 hours. All heated culture tubes which proved sterile had been incubated from 13 to 15 days. It should be stated that as nearly equally large amounts of emulsion as possible, were inoculated into each tube. The outstanding feature of this experiment is the direct proof of germination of the spores of B. botulinus in the animal body, as shown by the failure to obtain cultures from those tubes which were heated, when the same emulsion in unheated tubes gave a positive culture. This occurred 6 times and shows conclusively that these organs had either been invaded by vegetating forms or that the spores which they might have contained had germinated. No great difference is apparent between the cultures of the organs of those guinea-pigs which did and those which did not receive antitoxin.

After this encouraging result, we attempted to demonstrate in an even more conclusive manner the germination and possible multiplication of these organisms in the animal body. A tied loop or pocket was formed in the jugular vein of 3 rabbits. One-half to 1 c c of emulsion containing from 50,000,000 to 250,000,000 freshly detoxified spores was injected with aseptic precautions into this tied vein. After 48 to 69 hours this portion of the vein was excised without disturbing its contents, and a drop of liquid from the vein stained by the Gram method. The vein and contents from 2 rabbits was ground up with sterile saline and injected into mice with fatal results in 6½ and 17 hours. Microscopic preparations of this emulsion, as well as from the contents of the vein alone from all rabbits showed rare spores and a definite growth of gram-positive organisms with division (7 to 10 per microscopic field) which morphologically resembled B. botulinus. These emulsions immediately inoculated in equal amounts into deep agar (heated and unheated) gave a luxurious growth of typical colonies of B. botulinus in the unheated tubes, while those that were heated showed only rare

TABLE 11

RESULTS OF EXPERIMENTS WITH STRAIN 23, TYPE A—(FROM BROTH) RARE GRAM-NEGATIVE RODS

Serum Injections Subcutaneous

No.	Weight	Spores	Symptoms	Survival
Intravenous				
152	320	15 M + 0.5 c c N. serum	Botulism	Died after 45 hrs
153	369	15 M + 0.5 c c N. serum	Botulism	Died after 70 hrs.
154	388	15 M + 0.5 c c anti-toxin A	None	Killed after 47 hrs.
155	389	15 M + 0.5 c c anti-toxin A	None	Killed after 69 hrs.
Subcutaneous				
156	424	225 M	Botulism	Died after 6 days
157	470	225 M	Botulism	Died after 7 days

Positive Organ Emulsions		No. 152	No. 153	No. 154	No. 155
Lungs	Heated	+	+	0	0
	Unheated	+	+	+	+
Kidney	Heated	—	—	0	0
	Unheated	+	+	+	—
Mes.	Heated	+	+	0	0
	Unheated	+	+	+	+
Ing.	Heated	—	+	0	0
	Unheated	+	+	+	+
Bone	Heated	+	+	0	0
	Unheated	+	+	+	+
Spleen	Heated	+	+	+	+
	Unheated	+	+	+	+
Liver	Heated	+	+	+	+
	Unheated	+	+	+	+
Brain	Heated	—	—	—	—
	Unheated	—	0	—	+
Blood	Heated	—	—	+	0
	Unheated	—*	+	+	+
Gall	Heated	—	—	—	0
	Unheated	—	—	—	—

* Small amount inoculated; 0, cultures not taken.

colonies of the same organism. The rabbits died in from $3\frac{1}{2}$ to 6 days after injection. The centrifugalized blood serum of all the rabbits was fatal to mice. The emulsion of bone marrow and spleen of 2 rabbits were tested and only the spleen of 1 rabbit proved sufficiently toxic to produce death in a mouse in 36 hours. We also attempted to produce a growth from the spores of B. botulinus in the anterior chamber of the eyes of 2 rabbits. The fluid from the eye of 1 rabbit, removed after 76 hours, showed only a few gram-positive bacilli with division, but no spores. The fluid from the eye of the other rabbit, at the end of 20 hours, while most of the spores and bacilli had been removed by the intense leukocytosis which prevailed, thus duplicating the phagocytosis in the peritoneal fluid, enough gram-positive bacilli with end to end forms remained to show a definite germination and multiplication of the B. botulinus spores injected. Inoculation of this fluid directly into agar gave a culture of B. botulinus contaminated only by a few colonies of cocci. The fluid from the eye of each rabbit was fatal for mice (table 12).

We consider the results of these last two experiments as giving definite and conclusive evidence that the detoxified spores of B. botulinus actually germinate and multiply in the animal body. To just what extent this multiplication continues, we are not prepared to express an opinion. In the rabbits intravenously injected, death may have been caused by the transudation of the toxin through the vein wall into the circulating blood, or as the tied vein was not completely stripped of its minute collateral branches some of the bacteria or toxin may have escaped into the blood stream through these small vessels and produced more toxin there. In the same way, it is possible to explain positive cultures for some of the organs. The culture of these spores in a tied vein simulates closely the culture in vitro. The circulation of the blood is prevented or is exceedingly sluggish and more nearly resembles that of the lymphatic and retarded peripheral circulation of the blood which may be favorable to the growth of bacteria. In all events the growth, especially in No. 3, was most conclusive.

DISCUSSION

Whether a few spores remaining latent in the intestinal tract could ever find conditions favorable to extensive growth and consequent toxin production or invasion of the tissues seems extremely unlikely and remains for further quantitative cultural studies to decide. From the argument presented in considering the results of our cultural studies, it would appear that unless introduced in enormous numbers we have little to fear from the ingestion of toxin-free spores of B. botulinus, but we have shown that, when thus introduced, they cause symptoms and death from botulism. It has not yet been proved that the ingestion of small quantities of spores together with sublethal doses of toxin can produce symptoms or death from botulism, but from our few experiments with toxic wash water containing few spores, taken in conjunction with the experimental evidence obtained by other workers[34] along these lines, it is quite likely that under these conditions they may invade the tissues, proliferate and produce enough additional toxin to cause symptoms of the disease. The fact that the organs of fatally infected animals are generally microscopically sterile is partial evidence that the toxin is produced from exceedingly few vegetative forms.

TABLE 12

RESULTS OF EXPERIMENTS WITH STRAIN 23 — RARE VEGETATIVE FORMS: INJECTION OF HEATED SPORE SUSPENSION

Tied Vein of Rabbits

No.	Dose, C c	Amount	Vein Excised	Microscopic Vein Emulsion	Toxicity Vein	Survival, Days	Toxicity Organs for Mice	Cultures Organs
1	0.5	Approx. 50 M	69 hrs.	Rare spores, rare gram + bacilli division	....	6	Blood serum +	Liver, blood neg.
2	0.75	Approx. 100 M	48 hrs.	Rare spores, rare gram + bacilli division	+ typical 17 hrs.	4	Blood serum + Bone, spleen —	Blood neg. Spleen, bone +
3	1.0	Approx. 250 M	66 hrs. agar culture heated + unheated ++++	Rare spores, good growth, gram + bacilli division	+ typical 6½ hrs.	3½	Blood + Bone — Spleen + 36 hrs.	Blood neg. Bone, heated — unheated + Spleen, heated + unheated +
Anterior Chamber of Eye								
4	..	Not counted	Fluid withdrawn 76 hrs.	No spores, rare gram + bacilli division	Eye fluid + 24 hrs.	7	Blood serum +	Eye fluid — — Bone — Spleen, heated — unheated + Blood —
5	..	Not counted	20 hrs. agar culture heated + unheated ++++	Several spores, many gram + bacilli division	+ 28 hrs	2	Blood serum —	Eye fluid + Bone + Spleen — Liver + Blood —

When we consider the potency of the toxin of B. botulinus, this need not surprise us. It is probable that the spores do not find conditions equally favorable for germination in all the tissues of the body and that the vegetative forms which arise from the few spores which survive the enzymes or phagocytes are, in turn, quickly destroyed and their toxin liberated in the process. This may be why we have been able to recover the organisms in some cases from so few of the tissues of animals which have succumbed to spore injections.

We have no theory to offer as to delayed infection by latent spores for in none of our experiments has this occurred. Neither have we, by the usual procedures for other bacterial species, been able to produce such an infection after several days in animals which had shown no symptoms. Undoubtedly the spores or bacilli of B. botulinus, possibly in small numbers, remained latent, particularly in the liver and bonemarrow, for considerable periods after the animals had received massive doses. Whether these latent organisms, after once being established in equilibrium with the fluids and tissues of the body ever germinate or multiply later in sufficient numbers to produce the disease, is more or less of an open question. We are aware that botulism has occurred in animals which had been fed several months previously with spores and maintained afterward in cages grossly contaminated with dejecta or foods containing spores of B. botulinus, but we cannot be sure in these rare cases that there may not have been a continuous reinfection of the lungs and intestinal tract from the feces or food remaining in the cage. We have seen 1 normal animal in such a cage die of symptoms suspiciously resembling those of botulism.

Why some animals should succumb to botulism and others of the same weight receiving the same large dose under identical conditions should fail to show symptoms is another question which we have not attempted to answer. A natural immunity probably does not exist except under the conditions of the experiments, for we have later killed some of these surviving guinea-pigs with small doses of toxin. Neither in this case is it a question of the toxin having caused the germination or multiplication of latent spores or bacilli, for the normal controls died within the same period.

The mode of action or the paths of diffusion of the toxin of B. botulinus in the animal body has received no attention in our experiments, so that we have not sufficient information to discuss this phase of the question. Nor are we able to venture an opinion as to which of the tissues may play a preponderating rôle in the neutralization of the toxin as produced. The fact that emulsions of the organs of animals dying of botulism are so infrequently toxic leads us to suppose that the amount of toxin circulating in the system, at least postmortem, is very minute. It may be that at the time that the symptoms were produced and the damage done to the system it was in greater quantity and had been subsequently attenuated or destroyed by fixation in the tissues, or the process of this fixation may have been the condition causing the manifestation of symptoms.

While it is remotely possible, though we have no evidence to support the belief, that some of the toxin liberated in the animal body may be derived from a preformed endotoxin within the heated spore or bacterial cell, we have demonstrated beyond doubt that the toxin-free spores of B. botulinus do germinate and multiply in the fluids and tissues of the animal body and produce toxin there. Our cultural studies would tend to substantiate these conclusions. Therefore, in our opinion, the principal, if not the only, source of toxin in the body of our experimental animals is from the germination of these spores.

We wish to say in conclusion that our results showing the difficulty of infecting laboratory animals except by massive doses of heated B. botulinus spores should not be taken in any way as a criterion for what may happen in the human system by the consumption of spores of this organism in the natural way—although undoubtedly in the latter case enormous numbers if freed of their toxin would be required to produce infection.

CONCLUSIONS

Massive doses of toxin-free spores of B. botulinus are pathogenic when introduced into the animal body.

These spores and the vegetative forms arising from them are rapidly disseminated throughout the tissues of the body.

Toxin-free spores of B. botulinus germinate, and the vegetative forms arising from this germination multiply and liberate toxin in the animal body.

THE HEAT RESISTANCE OF THE SPORES OF B. BOTULINUS AND ALLIED ANAEROBES. XI

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As a part of a broad study dealing with the bacteriology and biochemistry of B. botulinus, the heat resistance of the spores of this and allied anaerobes has been investigated. A brief analysis of the accumulated data made on 1804 spore suspensions and 65 soil specimens naturally or artificially contaminated with the spores of B. botulinus, has revealed a number of important scientific and practical facts. As the detailed preparation and publication of the numerous experiments embracing over 42,807 cultures may be delayed for several months, it is deemed advisable to report the main tentative conclusions. The technic used in these studies is in principle identical with that employed by Bigelow and Esty[1] in their work on the heat resistance of thermophilic bacteria. In the course of the investigation minor modifications in the preparation of the cultures and the subcultures of the heated spore suspensions have been adopted in order to eliminate the danger of laboratory contaminations. Extensive experience has definitely proved the broad applicability and absolute reliability of this standard method.

Comparative Resistance of Sporulating Anaerobes to Moist Heat

(*a*) *B. botulinus.*—A comparative study of 109 strains of B. botulinus, which originated from numerous field specimens, soils and vegetables as well as food products responsible for 26 human and 9 chicken outbreaks of botulism, reveals two facts: (1) certain strains sporulate irregularly and poorly, and (2) the heat resistance fluctuates within wide limits. The spores employed in these tests have been produced in canned pea infusion—peptic-digest broth (equal parts P_H 8.4). After an incubation of 10 days at 35 C. the culture has been concentrated by centrifugalization and the sediment suspended in 50 c c of a M/15 phosphate solution of a P_H of 7.00 to 7.12 (Sorensen mixture of Na_2HPO_4 and KH_2PO_4). The mixture has been vigorously shaken in order to produce a uniform suspension, and 2 c c amounts have been delivered with the aid of a sterile buret into tubes of hard glass with a uniform bore, thickness, etc. These tubes have been sealed in an oxygen blast and heated at 105 C. (221 F.) in a DeKhotinsky electric oil bath. The temperature has been controlled by means of a thermo-regulator and kept uniformly distributed throughout

Received for publication, July 26, 1922.

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[1] Jour. Infect. Dis., 1920, 27, p. 602.

the bath by a turbine stirrer device. Subcultures of the heated spore suspensions have been made in a 0.5% glucose-beef-heart-peptic-digest broth (P_H 7.0-7.4) stratified with vaseline and incubated at 35 to 37 C.

The 109 strains are divided into 3 groups according to their toxicologic and serologic characteristics: type A, 78; type B, 30; nontoxic, 1 strain. Their relative heat resistance is shown in table 1.

At a temperature of 105 C. (221 F.), the average survival time of the spores of 78 strains of B. botulinus, type A, is 40.1 minutes, while the minimum is less than 9 minutes and the maximum 85 minutes. The survival time of the spores of 30 type B strains at the same temperature is on the average 23.7 minutes, with a minimum of less than 6 minutes and a maximum of 60 minutes. The average resistance of the 109 strains of B. botulinus is 35.2 minutes at 105 C. (221 F.). Spores of type B are apparently less heat resistant than those of type A strains. It is, however, evident that under exceptional circumstances strains and spores of a resistance equal to that recorded for type A strains may be encountered in nature or may be produced artificially in the laboratory.

TABLE 1

RELATIVE HEAT RESISTANCE OF THREE GROUPS OF B. BOTULINUS STRAINS

Resistance at 105 C. (221 F.) in Minutes	Number of Type A Strains	Number of Spores Heated	Number of Type B Strains	Number of Spores Heated	Total Number of Type A and B Strains
Less than 12......	3	{1 no count 0 }2 = 10 M.* and 100 M.	10	{1 no count 0 }100 M. to 1 B.	13
12 to 21...........	9	250 M. to 1,300 B.	5	200 M. to 3 B.	14
21 to 42...........	33†	25 M. to 3 B.	7	130 M. to 1,200 M.	40
42 to 60...........	16	130 M. to 3 B.	4	100 M. to 200 M.	20
60 to 90...........	18	250 M. to 12 B.	4	75 M. to 535 M.	22
Less than 12 to 90	79†	10 M. to 12 B.	30	75 M. to 3 B.	109

* M. = million; B. = billion.

† The spores of the nontoxic strain survived 30 minutes but were killed in 33 minutes. Two strains (1 A and 1 B) failed to produce spores either in pea peptic digest broth or brain medium; 9 strains (4 A and 5 B) were grown in minced sheep brain medium with a natural reaction after it was noted that pea peptic digest broth failed to yield a sufficient number of spores to make comparative tests. Spores of 4 strains cultivated and tested in this manner were destroyed in less than 12 minutes.

(*b*) *B. sporogenes.*—The heat resistance of 33 strains of B. sporogenes has been determined at 100, 105 and 110 C. The spores have been produced either in pea peptic digest, veal infusion-peptic-digest-gelatine or brain medium. The following heat resistances have been recorded:

100 C. (212 F.) 10 to 150 minutes; average survival time 72.5 minutes.
105 C. (221 F.) 4 to 45 minutes; average survival time 12 minutes.
110 C. (230 F.) 1 to 12 minutes; average survival time 3.3 minutes.

The cultures of heated spore suspensions exhibit the property of retarded germination. In several instances it has been noted that cultures failed to grow until after 100 days of incubation at 35 C.

(*c*) *B. tetani.*—The spores of 24 strains of B. tetani purified and described by H. H. Heller,[2] produced either in pea peptic digest broth, brain medium, double strength veal infusion-peptic-digest-gelatine or casein broth, exhibited the following heat resistance:

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[2] Ibid., 1922, 31, p. 31.

100 C. (212 F.) 15 to 90 minutes; average survival time 25 minutes.
105 C. (221 F.) 3 to 25 minutes; average survival time 9.2 minutes.

The maximum resistance was obtained in neutral brain medium. Germination of the heated spores in an optimum medium was not markedly retarded. All surviving spore suspensions showed growth within 6 days.

(*d*) *B. bifermentans.*—Three of the 4 strains of B. bifermentans (isolated and described by I. C. Hall[3]) survived 30 minutes, but were destroyed in 45 minutes at 100 C. The spores of one strain exposed to a temperature of 105 C. (221 F.) survived 18 minutes, but did not resist 21 minutes.

(*e*) *B. centrosporogenes.*—Three of 4 strains of B. centrosporogenes (isolated and described by I. C. Hall[3]) were destroyed in less than 30 minutes at 100 C. The fourth strain resisted 30 minutes but not 45 minutes at the same temperature.

(*f*) *B. welchii.*—One strain of B. welchii isolated from a heated soil specimen produced spores in sheep brain medium, which survived 24 minutes but not 27 minutes at 105 C. The spores of another strain were killed in 10 minutes but survived 5 minutes at 100 C.

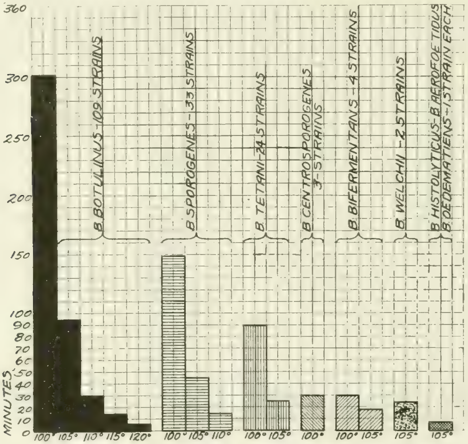

Chart 1. Maximum heat resistance of sporulating anaerobes.

(*g*) *B. histolyticus, B. oedematiens and B. aerofoetidus.*—One strain each of B. histolyticus, B. oedematiens and B. aerofoetidus failed to survive 6 minutes at 105 C. The anaerobe isolated by Bengtson[4] from the larvae of Lucilia Caesar was destroyed in less than 15 minutes at 100 C. The subcultures of the anaerobes mentioned under *d, e, f* and *g* exhibited no retarded germination.

The maximum heat resistances of the various anaerobes tested at different temperatures are shown in chart 1. It is definitely demonstrated that B. botulinus produces spores of extraordinary resistance toward moist heat.

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[3] Ibid., 1922, 30, p. 445.

[4] Pub. Health Rept., 1922, 37, p. 164.

Furthermore, a comparison of these data with those published by von Hibler,[5] Becker[6] and others indicates that the resistance of the spores of American strains of B. botulinus surpasses that of the sporulating anaerobes thus far investigated.

THE MAXIMUM RESISTANCE OF B. BOTULINUS

The maximum resistance to moist heat of B. botulinus spores artificially produced under optimum conditions of temperature, food, oxygen requirements, etc., and heated in a phosphate solution prepared from a mixture of M/15 Na_2HPO_4 and KH_2PO_4 to give a P_H value of approximately 7 is as follows:

4 minutes at 120 C. (248 F.)
10 minutes at 115 C. (239 F.)
33 minutes at 110 C. (230 F.)
100 minutes at 105 C. (221 F.)
330 minutes at 100 C. (212 F.)

The data of similar tests secured with suspensions containing billions of spores produced by the most resistant strains have been used in the compilation of the "ideal destruction curve" shown in chart 2. This curve includes the maximum resistance observed on 1,804 spore suspensions and shows the negative results obtained at the temperatures stated.

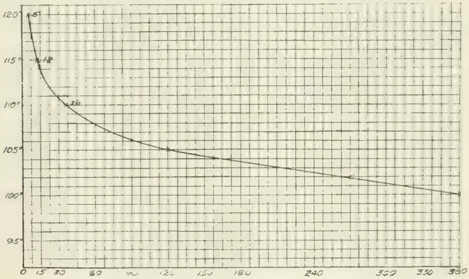

Chart 2.—Ideal destruction curve of B. botulinus strains 19, 23, and 97 grown in double strength veal peptic digest gelatine at 37 C. for 10 days, 60 billion in M/15 PO_4 solution, PH.7.0.

VARIATIONS IN THE HEAT RESISTANCE OF SPORES

The heat resistance of the spores of B. botulinus varies considerably, depending on several factors, some of which are unknown. The influence of the composition and the reaction of the broths used for the productions of the spores have been studied on 24 different mediums. The following preparations have been tested; pea-juice-peptic digest broth, pea-juice gelatine (10%), pea-juice-peptic-digest gelatine (10, 15 and 20%), pea juice, spinach juice, spinach juice with 2% Berna peptone, spinach-juice-peptic digest, spinach-juice-peptic-digest gelatine (10%), double strength veal infusion with and without peptic digest and gelatine, peptic-digest broth with and without Berna peptone (1%), diluted pea juice, glucose-peptic digest with and without gelatine, brain natural reaction and brain with 2% peptone, asparagus juice with 1% Difco-peptone, alkaline egg medium, casein digest, beef-heart-peptic-digest broth and milk agar in anaerobic plates (surface growth). In a number of mediums the influence of the P_H, the salt and phosphate content has been investigated. It has been established that the production of abundant spores with a fairly consistent heat resistance is regular in a double strength veal infusion-peptic-digest (equal parts) 10% gelatine with a P_H of 7.4.

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[5] Untersuchungen über pathogene Anaeroben, 1908.

[6] Centralbl. f. Bakteriol., O., I, 1920, 84, p. 71.

The spores which develop in certain mediums are always of very low resistance. Furthermore, it has been repeatedly noted that the spores generated in different flasks of the same medium, inoculated with equal amounts of the same stock culture and incubated for the same period, may show striking differences in heat resistance. These differences appear to be independent of the final reaction of the culture fluid within the range of P_H 6 to 8, the number and microchemical structure of the spores. Strains which sporulate poorly in liquid behave likewise in solid mediums.

TABLE 2

RESULTS OF EXPERIMENTS ON HEAT RESISTANCE OF SPORES

Flask	Final P_H	Millions of Spores Obtained and Heated	Resistance at 105 C. in Phosphate Mixture P_H 7.0		Retarded Germination
			Minutes +	Minutes —	
1	8.31	600	12	18	16 days
2	7.69	244	24	28	14 days
3	8.19	2,324	12	18	16 days
4	8.22	268	24	28	30 days
5	8.21	880	18	24	18 days
6	8.20	600	24	28	24 days
7	8.05	1,100	18	24	24 days
8	....	1,040	18	24	21 days

+ = survival time; — = destruction time.

One experiment is cited to illustrate this statement. Eight flasks of pea-peptic digest medium P_H 7.4 of the same lot were inoculated with strain 90 and each received 0.1 c c of the stock beef heart culture. The containers were incubated at 35 C for 10 days and the spores of each culture were heated separately in phosphate solutions of the same P_H prepared from the same standard mixture. The results are shown in table 2.

Supplementary tests have furthermore shown that striking variations are obtained when the spore suspensions are prepared and heated in the culture fluid in which they have been produced. For comparative tests it has, therefore, been found advisable to concentrate the spore material of several flasks containing from 800 to 1,500 c c of culture fluid by centrifugalization and to resuspend the sediment in a phosphate buffer solution of a neutral reaction. This phosphate mixture is easily duplicated, undergoes little or no change during the heating, and the spores tested in this solution have, as a rule, possessed the maximum resistance.

Several factors responsible for the peculiar variations in resistance deserve a more detailed discussion than is possible in this summary. They will be presented in the final report.

THE RESISTANCE OF B. BOTULINUS IN NATURAL AND ARTIFICIALLY INFECTED SOILS

For practical purposes it appeared advisable to determine the heat resistance of the spores of B. botulinus as found in nature. Suitable soil specimens selected from the collection of field samples which have been studied in connection with the problem on the distribution of this anaerobe in the United States have been used for this purpose. Definite amounts of 56 soil specimens originally collected in regions in which human or animal botulism had occurred and known to contain spores of B. botulinus have been weighed into large hard glass tubes containing 0.5% glucose-peptic digest-beefheart broth. These tubes have been sealed in an oxygen blast and heated in a bath of boiling water (99.5-100 C.) from 1 to 6 hours. The heated cultures have been incubated at 35 C. and observed daily. Growth has been judged by gas production, turbidity of the medium and the appearance of the meat. The presence of B. botulinus has been determined by toxin tests on mice and by deep agar shake cultures.

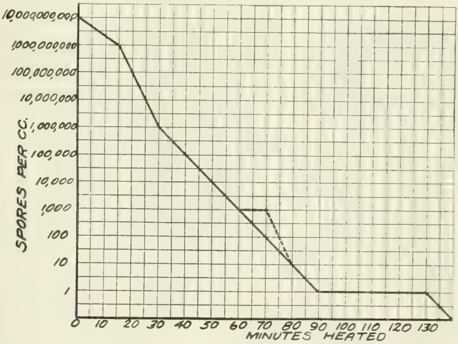

Chart 3.—Death rate of B. botulinus spores in phosphate M/15 KH_2PO_4 and NA_2HPO_4 at 100 C.

Three specimens of soil obtained from Colorado, New York and Washington contained a sufficient number of viable spores of B. botulinus to furnish toxic cultures after being heated. Two samples survived boiling for 1 hour and only 1 for 2 hours. All the samples heated for 150 minutes at 212 F. proved nontoxic and free from B. botulinus. These tests indicate that the average resistance of the spore of B. botulinus as found in approximately 20 gm. of naturally contaminated soil is less than 3 hours at 100 C. (212 F.).

Additional tests have been performed on 15 soil specimens, 6 sterile and 9 nonsterile, artificially inoculated with detoxified spores of B. botulinus and incubated for from 20 to 30 days aerobically or anaerobically at 27 C. It is known that B. botulinus developed in these soil cultures. Samples which contained sufficient toxin to kill guinea-pigs in from 20 hours to 5 days when fed, have been chosen for the heat resistance tests. Equal amounts of the soil suspension cultures have been treated in the manner stated. The data show that 8 of the 9 nonsterile soil samples developed toxic cultures after exposure to boiling water for 2 hours. All tubes heated for 150 minutes have been negative for B. botulinus. One sample of the 6 originally sterilized soil suspensions has become toxic after heating for 1 hour. The tubes heated for 2 hours have remained sterile. The spores of B. botulinus produced in sterile or nonsterile soil suspensions were destroyed in less than 3 hours. The second series of experiments fully confirm the results secured on naturally contaminated soil samples.

THE DEATH RATE OF B. BOTULINUS SPORES AT 100 C.

Chart 3 illustrates the death rate of the spores of B. botulinus suspended in a phosphate mixture and subjected to boiling water (99.5-100 C.). The survival of the spores has been determined in liquid mediums. Irrespective of the fact that the method furnishes only approximate data, it is evident that the mortality of the spores is a gradual process and follows in a general way the laws of logarithmic decline. The majority of spores are not exceptionally heat resistant. A relatively small number in this suspension survives the temperature of 100 C. for more than 90 minutes. Germination of these resistant spores may be delayed for several weeks or months. Uneven distribution of the resistant spores may favor the occurrence of so-called "skip-stops" (see paragraph The Influence of Concentration of Spores).

TABLE 3

RESULTS OF TIME-TEMPERATURE EXPERIMENTS

Strain	Grown in the Following Medium	Million Spores per 1 C.c.	P_H*	Heated in	Time in Minutes at									
					120 C.		115 C.		110 C.		105 C.		100 C.	
					+	-	+	—	+	—	+	—	+	—
62	Pea-peptic-digest broth	1,300	7.78	Pea-peptic digest 7.78 *	1	2	3	4	6	7	33	34	120	130
90	Pea-peptic-digest broth	1,800	8.01	Pea-peptic digest 8.01 *	1	2	3	4	9	10	44	48	160	170
47	Pea-peptic-digest broth	1,600	7.60	PO_4—6.85	1	2	6	7	15	16	65	70	285	300
97	Double strength veal infusion-peptic-digest gelatine	19,200	7.56	PO_4—6.88	4	5	9	10	28	30	90	100	315	330

* Final reaction of medium at the end of growth (10 days at 35 C.).
+ = survival time; — = destruction time.

THE TIME-TEMPERATURE RELATIONSHIP

The time necessary to destroy a known suspension of spores in a medium of known hydrogen-ion concentration decreases as the temperature increases. This fact has been established on 7 different spore suspensions. The spores employed in the experiments have been grown either in pea-peptic-digest broth, or in double strength veal infusion-peptic digest gelatine; the suspensions have been heated in the same medium in which the spores have been produced or in phosphate solutions. The results of 4 series are shown in table 3 and chart 4. The curves in chart 4 show the destruction times at the different temperatures.

THE INFLUENCE OF AGE OF THE SPORES ON THE THERMAL DEATH RATE

Four series of experiments have been made to determine the influence of age on the heat resistance of spores held at 35 C., 28 C. and in the icebox (average, 5 C.). In series 1, spore suspensions produced in pea juice-peptic digest broth have been tested after 4, 8, 12, 16, 20 and 30 days' incubation at 35 and 28 C. The spores of a culture kept at 35 C. for 30 days are less resistant than those incubated for only 4 days. This difference is, however, less marked for spores kept at 28 C. Spore production is slower at 28 C. The maximum resistance is reached on the 8th day and remains fairly consistent when kept at 28 C. The deterioration of the resistance is not influenced by the number of spores. In this series the average count remained practically constant irrespective of the low resistance of the old spores kept at 35 C.

In series 2, the influence of age has been determined by growing strain 90 in pea-peptic digest broth, with and without gelatine, and in neutral brain medium at 37 C., and 28 C. Resistance tests have been made in from 1½ to 186 days. The maximum resistances have been obtained in 1½ to 3½ days in pea gelatine, 2½ days in pea-peptic digest and 6 to 10 days in brain medium at 37 C. After these periods deterioration of the resistance is rapid, although the number of spores present in the medium remains fairly constant. At 28 C. the maximum resistance in pea gelatine has been reached in 3½ days, and in peptic digest broth and brain medium in 8 days. After 15 to 20 days the resistance has dropped rapidly in all cultures irrespective of the number of spores per cubic centimeter of medium.

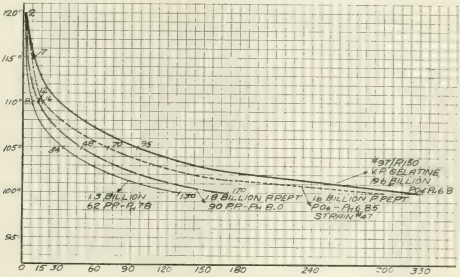

Chart 4.—Destruction curves.

The third series determined, in duplicate sets, the maximum resistance of spores produced in double strength veal-infusion-peptic digest-10%-gelatine broth at 37 C. Samples have been taken from the same flasks on the 2nd, 4th, 6th and 10th day. Abundant spores have been noted from the 4th day on, and the resistance has remained constant when tested on the 6th and 10th day.

The fourth series confirmed the previous observations. Furthermore, it demonstrated that cultures or spore suspensions kept in the icebox (5 C.) retain their maximum resistance for at least 30 days. Deterioration is, however, noted after a storage of 60 days. Cultures in double strength veal infusion-peptic digest-10%-gelatine broth containing excellent spores exhibit a gradual decrease in resistance after 21 days when kept at 37 C. This deterioration increases on incubation and is quite marked after 60 days. These experiments definitely indicate that the young spores, probably those of the first generation, are the most heat resistant. A well buffered medium, which furnishes abundant food material for progressive growth, produces, as a rule, spores of average or low resistance. Furthermore, any factor, as, for example, body temperature, which favors after the 10th day the continuous development of new spore-cycles, is conclusive to the production of abundant but poorly resistant spores. Spore suspensions in the moist state are preserved in the icebox for periods not exceeding 30 days.

THE RESISTANCE OF DRIED SPORES

On account of the great variability in the heat resistance of moist spores produced under ideal conditions, it is advisable to employ dried spores for a comparative study of the various factors which are ultimately responsible for their death. Numerous experiments have been conducted to standardize the procedures of dehydration of moist spore suspensions. In brief, it has been found that dried spores are somewhat less resistant than moist spores. Some of the factors responsible for this difference are in the course of investigation. The methods employed to prepare dried spores are briefly as follows: Washed spore suspensions are mixed with sterile sand. The moist material is smeared in Petri dishes and dried in vacuo over H_2SO_4 $CaCl_2$ or P_2O_5. Rapid drying in from 4 to 6 hours in vacuo at 34 C. (93 F) has produced the best results, and spores with a fairly high degree of heat resistance have been secured. Over a period of 347 days the resistance has remained constant and it appears that perfectly dry spores can be preserved equally well at 37 C., room temperature (20 C.), or in the icebox. Dried spores were found to be excellently suited for comparative studies, and their use can be highly recommended.

TABLE 4

RESULTS OF TESTS MADE TO DETERMINE RELATIONSHIP OF CONCENTRATION OF SPORES TO HEAT RESISTANCE

Strain	Number of Spores per 1 C c	Resistance in Min. at 105 C.		Resistance in Min. at 100 C.	
		+	—	+	—
90	900,000,000	44	48	...	...
	9,000.000	34	36	...	...
	90.000	18	20	...	...
	900	12	14	...	...
	9	..	2	...	...
90	628,000,000	33	36	...	...
	6,280,000	33	36	...	...
	62,800	24	27	...	...
	6,280	15	18	...	...
	628	10	15	...	...
	63	..	5	...	...
97	72,000,000,000	..	..	230	240
	1,640,000,000	..	..	120	125
	32,800,000	..	..	105	110
	650,000	..	..	80	85
	16,400	..	..	45	50
	328	..	..	35	40

+ = survival time; — = destruction time.

THE INFLUENCE OF CONCENTRATION OF SPORES

The relationship of the concentration of the spores to the heat resistance has been determined on 15 different spore suspensions. The results secured in 3 tests are shown in table 4.

From the foregoing data it is evident that the greater the concentration, the greater the resistance. On account of the remarkable variability of the resistance of B. botulinus spores produced of one and the same strain under identical conditions, this condition holds true only for the dilutions of a given suspension. It is a common experience to encounter cultures with less than one million spores, although their resistance surpasses that of others containing several billion organisms per c c.

In this connection, attention is again called to the phenomenon of retarded germination and the occurrence of so-called "skip-stops" within the first 90 days of incubation. Subcultures prepared from heated suspensions have germinated after an incubation of 378 days at 36-37 C. The cultures produced a virulent toxin and presented typical morphologic and biochemical characteristics.

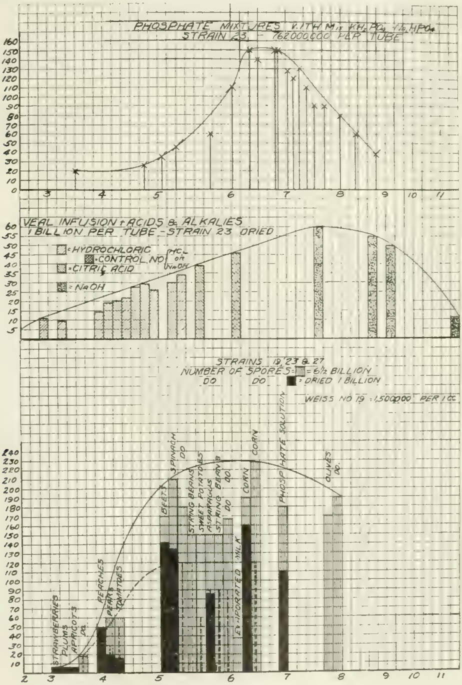

Chart 5.—Resistance of B. botulinus spores in phosphate mixtures, veal infusion of varying PH values by the addition of acid or alkali and food juices.

It has been frequently observed that subcultures made from suspensions which have been heated for long periods presented growth sooner than those heated for shorter times. These so-called "skip-stops" are believed to be the result of an uneven distribution of a relatively small number of very heat resistant spores. The occurrence of these "skip-stops" can, in part, be reduced by the preparation of carefully strained, uniform suspensions and by prolonged incubation. Subcultures prepared from diluted unheated or slightly heated spore suspensions (80 degrees for 1 hour or 100 degrees for 2 minutes) exhibit the phenomenon of retarded germination and "skip-stops." These and similar observations obviously indicate that the germination of a few spores of B. botulinus and B. sporogenes in suitable culture mediums is irregular and sometimes retarded for considerable periods.

The Effect of the P_H on the Thermal Death Rate

The effect of the hydrogen and hydroxyl-ion concentration on the heat resistance of spores of B. botulinus has been determined by suspending carefully washed spores in 4 different series of solutions of varying P_H values: (a) phosphate mixture series (M/15 Na_2HPO_4 and KH_2PO_4) varying from P_H 3.50 to 8.80; (b) 2% Difco peptone solution; (c) double strength veal infusion, and (d) spinach juice (extract of fresh leaves in boiling tap water for 1 hour) with varying amounts of N/10 acid or alkali.

Chart 5 illustrates the heat resistance of spores in phosphate mixtures. It is evident that the maximum resistance is on the acid side (about P_H 6.3 to 6.9. Final conclusions cannot be drawn from this series since prolonged incubation may change the available data.

TABLE 5

Effect of Heat on Spores of B. botulinus Suspended in Acidified Spinach Juice

Hydrochloric Acid				Citric Acid			
P_H	Resistance at 100 C. in Minutes		Retarded Germination, Days	P_H	Resistance at 100 C. in Minutes		Retarded Germination, Days
	+	—			+	—	
Control 5.05	45	50	7	Control 5.26	60	65	10
4.80	45	50	10	4.92	45	50	29
4.50	40	45	8	4.77	40	45	10
4.38	35	40	58	4.69	35	40	9
4.31	24	26	20	4.54	30	35	7
4.16	20	22	8	4.50	30	35	29
4.11	15	20	7	4.37	25	30	8
3.98	10	15	4	4.34	20	25	9
3.81	10	15	5	4.31	20	25	9
3.70	10	15	10	4.31	18	20	9

= survival time; — = destruction time.

The effect of 0.003 to 0.05 normal hydrochloric and citric acid and of 0.015 to 0.074 normal sodium hydroxide in double strength veal infusion on the spores is also shown in chart 5. The influence of the hydrogen-ion concentration on the heat resistance is marked below P_H 5.0 and above P_H 10.0 Malic, acetic, succinic and tartaric acid produce in a general way the same effect; below P_H 5.0 the resistance is greatly decreased. Hydrochloric and acetic acids are distinctly more toxic, while succinic, citric, malic and tartaric act in the order named. The behavior of the spores in acid or alkaline peptone solutions is similar to that in veal infusion.

The effect of heat on the spores of B. botulinus suspended in acidified spinach juice is shown in table 5. Definite amounts of hydrochloric and citric acids varying from 0.00025 to 0.027 normal were added to the spinach juice obtained as stated to produce a series of P_H values varying from 5.05 to 3.70 for hydrochloric acid and of P_H 5.26 to 4.31 for citric acid. This juice is rich in buffer substances and remained unaltered until 0.003 normal acid was added.

In the P_H range 4.5 to 5.5 little or no effect of the acid is noted; below 4.5 the decrease in the resistance is marked, as shown in table 5. It has been impossible to test the effect of alkalies on account of the profound chemical changes produced in the spinach juice by the addition of sodium carbonate or hydroxide.

THE EFFECT OF SODIUM CHLORIDE AND POTASSIUM CHLORIDE ON THE THERMAL DEATH RATE

Dried spores of several strains have been exposed to varying concentrations of sodium chloride in double strength veal infusion at different temperatures. No reduction in the heat resistance has been noted until the concentration has reached 8% sodium chloride. Ten and 20% solutions of sodium chloride decrease the resistance, while, on the other hand, the addition of 0.5 and 1% sodium chloride makes the destruction more difficult than in a 2 or 3% solution. These observations are briefly summarized in table 6.

An extensive series of tests has been carried out to determine the effect of holding vegetative forms and spores in 20% sodium and potassium chloride solutions with and without double strength veal infusion and Difco peptone (2%). It is shown that the Na-ion is more toxic than the K-ion, since the resistance is conclusively much less in sodium than in potassium solutions.

TABLE 6

EFFECT OF SALT ON THERMAL DEATH RATE

Spores Suspended in	P_H	Resistance at 100 C. in Minutes	
		+	—
Veal infusion— 0.0 NaCl	6.03	35	40
Veal infusion— 0.5% NaCl	5.94	45	50
Veal infusion— 1.0% NaCl	5.94	50	55
Veal infusion— 2.0% NaCl	5.83	30	35
Veal infusion— 4.0% NaCl	5.75	30	35
Veal infusion— 5.0% NaCl	5.70	35	40
Veal infusion— 6.0% NaCl	5.65	20	35
Veal infusion— 8.0% NaCl	5.51	25	30
Veal infusion—10.0% NaCl	5.50	20	24
Veal infusion—20.0% NaCl	5.06	10	12
M/15 phosphate mixture	6.92	105	110

+ = survival time; — = destruction time.

Spore suspensions held in 20% salt solutions become more resistant to heat during an incubation of 60 days at 37 C. Spores of considerable resistance are present even after an incubation of 340 days at this temperature.

Spores heated in peptone solutions without salts or meat extractives exhibit a relatively low heat resistance, as is well illustrated by the data in table 7.

The influence of certain amino groups, polypeptides, on the heat resistance of spores is suggested by these observations and deserves for numerous reasons careful investigation.

THE RESISTANCE OF B. BOTULINUS SPORES IN FOOD JUICES

The heat resistance of 4 different spore suspensions of B. botulinus has been determined in the following juices of 17 varieties of canned food: corn, spinach, asparagus, string beans, peas, ripe olives, evaporated milk, beets, sweet potato, tomato, sauerkraut, pears, peaches, apricots, plums, strawberries and cherries. The spores have been washed twice in sterile tap water, resuspended in tap water, and 5 c c of the suspension has been added to 45 c c of the food juice.

In chart 5 the results recorded with 2 different spore suspensions are shown in graphic form. It will be noted that above P_H 5.0 some factor other than hydrogen-ion concentration has a profound influence on the time necessary for the sterilization of foods. In this respect the results differ fundamentally from those previously determined on phosphate mixtures or veal infusion acid and alkali solutions, as shown in chart 5. For example, it is striking that spores heated in ripe olives with a P_H 7.93, corn P_H 6.35 and spinach P_H 5.05 have approximately the same resistance, while, on the other hand, in asparagus with a P_H 5.25 and 5.55 sterilization is more readily accomplished. In food juices with a P_H value below 4.5 the hydrogen-ion concentration has a marked influence on the destruction of B. botulinus spores. Careful consideration should be given to these facts in estimating the processing times and temperatures applicable to the practical canning of food.

TABLE 7

Heat Resistance of Spores in Various Solutions

Spores Suspended in	P_H	Resistance at 100 C. in Minutes	
		+	—
Phosphate solution	6.92	110	120
Peptic digest broth	7.00	120	130
Double strength veal infusion	6.70	90	100
2% aminoids	5.65	60	65
	5.65	60	65
2% Difco peptone	7.40	40	50
	7.08	30	35
2% Witte's peptone	7.45	20	25
	7.45	20	25

SUMMARY

The heat resistance of 109 strains of B. botulinus (78 type A, 30 type B and one nontoxic) from various sources varies from three to 80 minutes at 105 C. The spores were produced in pea peptic digest broth, P_H 8.0, and heated in a phosphate solution P_H 7.00-7.12.

The heat resistance of 33 strains of B. sporogenes varies from 10 to 150 minutes at 100 C.; four to 45 minutes at 105 C. and one to 12 at 110 C.

The heat resistance of 24 strains of B. tetani varies from 15 to 90 minutes at 100 C. and three to 25 minutes at 105 C.

Strains of B. bifermentans, B. centrosporogenes, B. histolyticus, B. oedematiens, B. aerofoetidus and the anaerobe isolated by Bengston are killed within six minutes at 105 C. One strain of B. welchii survived 24 minutes at 105 C. but was destroyed in 27 minutes.

The maximum heat resistance of B. botulinus spores artificially produced under the most favorable conditions for growth and heated in a phosphate solution of a P_H 7.0 is as follows:

4 minutes at 120 C. (248 F.)
10 minutes at 115 C. (239 F.)
32 minutes at 110 C. (230 F.)
100 minutes at 105 C. (221 F.)
330 minutes at 100 C. (212 F.)

These results represent actual survival times at the given temperatures.

The heat resistance of spores of the same strain of B. botulinus varies considerably, depending on several factors, some of which are unknown.

The average resistance of the spores of B. botulinus, as found in approximately 20 grams, either of naturally or artificially contaminated soil is less than three hours at 100 C. (212 F.).

The death rate of B. botulinus spores is gradual and follows in a general way the laws of logarithmic decline. The majority of the spores are not exceptionally heat resistant.

Young moist spores, probably those of the first generation, appear to be the most heat resistant. Spore suspensions in the moist state are best preserved in the icebox for periods not exceeding 30 days. Prolonged incubation of spores held at 20 C. or 35 C. tends to lessen the resistance.

The heat resistance of dried spores has remained constant over a period of 347 days, and it appears that perfectly dry spores can be preserved equally well at 37 or, 20 C. or in the icebox.

It is evident that the heat resistance increases as the concentration increases determined by dilutions of a given suspension. However, cultures with less than one-million spores may have a greater heat resistance than others containing several billion organisms per c c.

Subcultures from heated spore suspensions of B. botulinus have germinated after an incubation of 378 days at 36-37 C.

The hydrogen ion concentration of different solutions affects the thermal resistance of B. botulinus spores.

Spores heated in concentrations of sodium chloride from 0.5% to 6% do not decrease in heat resistance. The presence of 8, 10 and 20% solutions greatly decreases the resistance while 0.5% and 1.0% appear to enhance it. Spores heated in peptone solutions without salts or meat extractives exhibit a relatively low heat resistance.

The heat resistance of B. botulinus spores in the juices of 17 varieties of canned food shows a variation from less than 10 minutes to 230 minutes at 100 C.

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